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Phosphorylation of Photosynthetic Antenna Protein CP29 and Photosystem II Structure Changes in Monocotyledonous Plants under Environmental Stresses†

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ABSTRACT: Kinetic studies of protein dephosphorylation in thylakoid membranes showed that the minor light-harvesting antenna protein CP29 could be phosphorylated in barley (C3) and maize (C4) seedlings, but not in spinach under water [Liu, W. J., et al. (2009) *Biochim. Biophys. Acta 1787*, 1238−1245], salt, or cold stress [Pursiheimo, S., et al. (2003) *Plant Cell Environ. 26*, 1995−2003], suggesting that phosphorylation of CP29 is a general phenomenon in monocots, but not in dicots under environmental stresses. Abscisic acid (ABA), reactive oxygen species (ROS), salicylic acid (SA), jasmonic acid (JA), ethylene (ET), NO, and the scavenger of H_2O_2 had weak effects on CP29 phosphorylation. However, three protein kinase inhibitors, U0126, W7, and K252a (for mitogen-activated protein kinase, Ca^{2+}-dependent protein kinase, and Ser/Thr protein kinases, respectively), decrease the level of CP29 phosphorylation in barley apparently under environmental stresses. Therefore, these three protein kinases are involved in CP29 phosphorylation. We also found that most CP29 phosphorylation was accompanied by its lateral migration from granum membranes to stroma-exposed thylakoid regions, and the instability of PSII supercomplexes and LHCII trimers under environmental stresses.

Photosystem II (PSII)[1] is multisubunit, membrane-spanning complex responsible for a water-plastoquinone oxidoreduction in the thylakoid membrane of algae, cyanobacteria, and higher land plants. In higher plants, the antenna system surrounding PSII is composed of at least six different chlorophyll *a*/*b* binding light-harvesting complexes (LHCs) (*1*). The PSII outer antenna complex consists of the major components (different heterotrimers of *LHCB1*, *LHCB2*, and *LHCB3* gene products) and minor antenna complexes CP29, CP26, and CP24, encoded by *LHCB4*, *LHCB5*, and *LHCB6* genes, respectively (*1*, *2*).

Many proteins in photosynthetic membranes of plant chloroplasts can be phosphorylated. CP29, which is encoded by three highly homologous *Arabidopsis* genes (*3*) and lies between the major antenna complexes and the PSII reaction center, has specific roles in light harvesting, energy dissipation, and the macro-organization of supercomplexes in the thylakoid membranes (*4*). Among three minor antennae, CP29 is the only phosphoprotein in higher plants, and its phosphorylation is involved in photoinhibition recovery (*5*) and state transitions (*6*, *7*). The detailed phosphorylation site of CP29 in *Arabidopsis thaliana* and green alga was identified by mass spectrometry (*8*, *9*). Phosphorylation of CP29 depends on strong reduction of the plastoquinone pool and was further enhanced by low temperatures (*10*). In vitro studies demonstrated that CP29 phosphorylation is independent of the redox state of both the cytochrome b_6/f complex and the thiol compounds (*10*). On the other hand, phosphorylation of Lhcb1 and Lhcb2 occurred only upon modest plastoquinone reduction and was subject to inhibition with an increase in the thiol redox state of plastid stroma (*10*−*12*). In *Arabidopsis*, STN8 kinase is essential for PSII core protein phosphorylation, whereas STN7 is required for LHCII phosphorylation, including CP29 (*10*−*12*). Although several protein kinases controlling thylakoid protein phosphorylation and the differences in phosphorylation conditions between PSII core and LHCII proteins have been well documented, the molecular mechanisms of these regulatory events remain largely unknown.

Phosphorylation and dephosphorylation of thylakoid proteins are important biochemical processes in plant cells. They play an important role in PSII structure changes and repair cycles of PSII. Following illumination, PSII supercomplexes and LHCII trimers disassemble. Several PSII core proteins and LHCIIs undergo phosphorylation under this condition and then laterally migrate from granum membranes to stroma lamellae for degradation and de novo synthesis (*13*, *14*). Our previous study, however, showed that this classical model is not necessarily in use in monocotyledonous plants, such as barley. Our previous research suggested that, in barley under water stress, phosphorylation and lateral migration from granum membranes to stroma-exposed lamellae were found only in CP29, and the other PSII proteins did not migrate and were degraded directly in situ (*15*). In this work, we further investigated some other

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[1]Abbreviations: CDPK, Ca^{2+}-dependent protein kinase; Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea; SNP, sodium nitroprusside; DM, *n*-dodecyl β-D-maltoside; DMTU, *N*,*N*′-dimethylthiourea; LHCII, light-harvesting complex II; MAPK, mitogen-activated protein kinase; PSII, photosystem II; q_P, photochemical quenching; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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environmental stresses, such as salt stress, low temperature, and high light, and compared them to the pattern observed during water stress. The protein kinases for CP29 phosphorylation, the related PSII structure changes, and PSII protein migration are discussed.

MATERIALS AND METHODS

Plant Materials and Stress Treatment. Barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), and spinach (*Spinacia oleracea* L.) were grown in sand and cultured with 1/2 Hoagland solution at 25 ± 1 °C under a 12 h photoperiod and a photosynthetic photon flux density of 100 μmol of photons m^{-2} s^{-1}. Barley and maize seedings from 2-week-old plants and 2-month-old spinach were removed from the sand, washed with tap water, and dried briefly with paper towels to remove surface water. Water stress was initiated by submersion of the roots in a nonpenetrating PEG solution (aerated with air) (*16*) with an osmotic potential of −0.5 MPa in beakers. Salt stress was introduced in 0.3 mol/L NaCl, and cold treatment was at 4 °C (100 μmol of photons m^{-2} s^{-1}). High-light treatment (1000 μmol of photon m^{-2} s^{-1}, 2 h) was given after dark adaption for 16 h. To investigate the mechanism of CP29 phosphorylation, 2-week-old plants (before environmental stresses) were pretreated with 25 μM U0126 (MAPK inhibitor) (*17*), 100 μM W7 (CDPK inhibitor) (*18*), 50 μM H-89 (cAMP-dependent protein kinase inhibitor) (*19*), 20 μM K252a (Ser/Thr protein kinases inhibitor) (*19*), or 5 mM dimethylthiourea (DMTU, a ROS scavenger) (*17*) for 24 h and then exposed to the same stress conditions as described above. Some other plants were treated with 100 μM jasmonic acid (JA), 0.2 mM salicylic acid (SA), 150 mg/L ethephon (ET, a precursor of ethylene), 0.5 mM sodium nitroprusside (SNP, a donor of NO), 100 μM abscisic acid (ABA), 20 mM H_2O_2, 20 μM 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB, an electron transport inhibitor), or 20 μM 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea (DCMU, another transport inhibitor) under the control growth conditions for 24 h (*10*). Incorporation efficiencies of DCMU and DBMIB (by vacuum infiltration) were tested by measuring leaf $1 - q_P$ values in low light (100 μmol of photons m^{-2} s^{-1}), which were 0.91 for DCMU and 0.48 for DBMIB (the $1 - q_P$ value of control leaves was 0.11). Incorporation efficiencies of protein kinases inhibitors were tested by measuring corresponding protein kinase activities (data not shown).

Isolation and Subfractionation of Thylakoid Membranes. Isolation and subfractionation of thylakoid membranes were conducted immediately after different treatments. PSII membranes were prepared according to the protocol developed by Suorsa et al. (*20*) with 20 mM NaF. Then the thylakoid membranes were mechanically broken by being shaken (without sonication) and then purified with an aqueous two-phase system to isolate the grana and stroma lamellae fractions, and the grana fraction was further purified to isolate the grana core and the grana margin fractions by sonication, using the methods of Jansson et al. (*21*) and Danielsson et al. (*22*).

SDS−PAGE, Protein Staining, and Western Blot Analysis. According to the method of Tikkanen et al. (*7*), isolated thylakoids were solubilized in the presence of 6 M urea, and the polypeptides were separated by SDS−PAGE using 15% (w/v) acrylamide gels with 6 M urea. For Western blotting, the proteins were electrotransferred onto a nitrocellulose film using the method of Yuan et al. (*23*). Then antisera to D1, PsaA, LHCIa1, and LHCII b1 and b4 (purchased from Agrisera, Umea, Sweden) were applied. The signals were revealed by using secondary antibodies of alkaline phosphatase goat anti-rabbit IgG.

CP29 Immunoprecipitation. ^{32}P labeling of thylakoid membrane proteins was performed by illumination (100 μmol of photons m^{-2} s^{-1}) in the presence of 0.25 mM ATP containing [γ-^{32}P]ATP for 45 min at room temperature. Then thylakoid membrane proteins were isolated and immunoprecipitated with the CP29 antibody and analyzed after β-mercaptoethanol treatment by SDS−PAGE and immunoblotting for CP29 or autoradiography (*24*, *25*).

Blue Native Polyacrylamide Gel Electrophoresis Analysis of Thylakoid Membrane Protein Complexes. Blue native polyacrylamide gel electrophoresis (BN-PAGE) was conducted as described in refs *26* and *27* with some modifications. The thylakoid membranes were washed in 330 mM sucrose and 50 mM BisTris-HCl (pH 7.0) and suspended in resuspension buffer [20% (w/v) glycerol and 25 mM BisTris-HCl (pH 7.0)] at a concentration of 1.0 mg of Chl/mL. An equal volume of resuspension buffer containing 2% (w/v) *n*-dodecyl β-D-maltoside (DM) was dropped into the thylakoid suspension. After incubation at 4 °C for 10 min, insoluble material was removed by centrifugation at 15000*g* for 15 min. The supernatant was combined with $^1/_{10}$ volume of 5% Serva blue G in 100 mM BisTris-HCl (pH 7.0), 0.5 M 6-amino-*n*-caproic acid, and 30% (w/v) glycerol and applied to 0.75 mm 4−13% acrylamide gradient gels. Electrophoresis was performed at 4 °C and 10 mA for 3 h. Then Western blots with anti-D1, anti-LHCII b1, and anti-CP29 for BN gels were performed.

Fluorescence Measurements. The redox state of the Q_A electron acceptor of PSII ($1 - q_P$) was monitored by measuring the photochemical quenching of chlorophyll fluorescence (q_P) with a PAM-2100 fluorometer (Heinz Walz, Effeltrich, Germany). After the leaf had been transferred to the PAM fluorometer, steady-state fluorescence (F_s) was attained within 30 min at the same light intensity and temperature that prevailed during the experimental treatment of plants. The minimal fluorescence level in the dark-adapted state (F_o) was measured using the modulation light, which was sufficiently low (< 0.1 μmol m^{-2} s^{-1}) not to induce any significant variable fluorescence. We measured the minimal fluorescence level in the light-adapted state (F_o') after turning off 100 μmol m^{-2} s^{-1} PFD of the actinic light, which was equivalent of the growth light intensity, and illuminating the sample with far-red light for 3 s. The $1 - q_P$ value was measured as $(F_s - F_o')/(F_m' - F_o')$ (*10*).

Statistical Analysis. All Western blots and BN-PAGE were repeated twice, and typical results are presented. Then the quantitative data of Western blots and BN-PAGE were analyzed and are presented in the Supporting Information. A Student's *t* test was used for comparison between different treatments. A difference was considered to be statistically significant when $p < 0.05$.

RESULTS

Stress-Induced Phosphorylation of CP29 in Barley and Maize, but Not in Spinach. The CP29 phosphorylation level could be easily detected by a normal Western blot, which shows two bands, while the upper band indicates the phosphorylated CP29. The bands of phosphorylated CP29 were further confirmed by ^{32}P labeling, immunoprecipitation, and then autoradiography (Figure 1). It is well-known that CP29 could be phosphorylated in maize and barley when they are exposed to cold treatment (*5*, *25*). Our results further indicated that CP29

could be phosphorylated under water, cold, or salt stress in two monocotyledonous plants, barley (C3 plant) and maize (C4 plant), but not in dicotyledonous plants, spinach (Figures 1 and 2) and *Arabidopsis* (data not shown). However, under the high-light condition, all plants had phosphorylated CP29 (Figure 2). It could be suggested that monocotyledons and dicotyledons have different patterns of phosphorylation of CP29 under environmental stresses (except high-light stress).

Redox State of the Plastoquinone Pool. Previous studies suggested that phosphorylation of CP29 occurred with a strong reduction of the plastoquinone pool (*10*). Therefore, we detected photochemical quenching (q_P) under low light (100 μmol of photons m^{-2} s^{-1} at 20 °C). Under slight water or salt stress, $1 - q_P$ hardly increased, while it tripled under severe water or salt stress. In contrast, high-light stress for 2 h (1000 μmol of photons m^{-2} s^{-1}) or cold stress for 1 day increased $1 - q_P$ rapidly (Table 1). These data imply that rapid CP29 phosphorylation under slight water or salt stress was not related to the redox state of the plastoquinone pool. There should be some other regulatory mechanisms besides the plastoquinone pool.

MAPK, CDPK, and Ser/Thr Protein Kinases Charge CP29 Phosphorylation in Monocotyledonous Plants. What is the regulatory mechanism other than the plastoquinone pool?

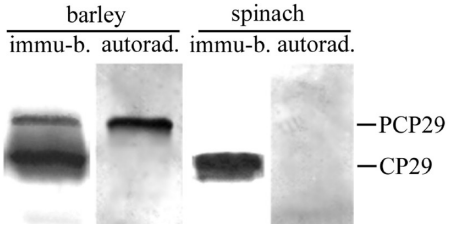

FIGURE 1: Detection of CP29 phosphorylation. ^{32}P-labeled and solubilized thylakoid membrane proteins from cold-stressed barley or spinach seedlings were immunoprecipitated with CP29 antibodies and analyzed after β-mercaptoethanol treatment by SDS−PAGE and immunoblotting with CP29 (immu-b.) or autoradiography (autorad.).

We selected several typical compounds related to environmental stress (ABA, SA, JA, H_2O_2, ET, and NO) and investigated their effects on CP29 phosphorylation. The results indicated that exogenous ABA, SA, JA, H_2O_2, ET, and NO had no obvious effect on CP29 phosphorylation (Figure 3A) while the presence of DBMIB (an electron transport inhibitor, which induces reduction of the plastoquinone pool) apparently induced CP29 phosphorylation, which could be completely reversed by DCMU (another electron transport inhibitor, which induces oxidation of the plastoquinone pool) (Figure 3A). These results indicate that CP29 phosphorylation is controlled by the redox state of plastoquinone but does not correlate with common stress response chemicals.

Then the candidate protein kinases involved in CP29 phosphorylation were studied. Pretreatment with the MAPK inhibitor U0126 decreased the level of water stress or salt stress-induced CP29 phosphorylation markedly, especially at slight stresses. W7, the inhibitor of CDPK, had a similar effect. However, for cold stress and high-light stress, MAPK and CDPK inhibitors could not decrease the levels of phosphorylated CP29. H-89 (cAMP-dependent protein kinase inhibitor) had no obvious effect on CP29 phosphorylation under any stress conditions. On the other hand, the Ser/Thr protein kinase inhibitor K252a largely inhibited all CP29 phosphorylation, including the phosphorylation under cold stress or high-light stress (Figure 3B,C and Figure S1 of the Supporting Information). Pretreatment with DMTU partly downregulated CP29 phosphorylation under all stress conditions. Considering that H_2O_2 is not related to CP29 phosphorylation, the downregulation by DMTU may be due to fact that ROS scavengers alleviate the stress damages (*17*) and therefore alleviate plastoquinone over-reduction. These results suggest that MAPK and CDPK are most possibly involved in the modulation of CP29 phosphorylation, although the role of the plastoquinone redox state is dominative.

Lateral Migration of CP29 during Different Stresses. To investigate the effects of three different stresses on the distribution of PSII proteins, we fractionated thylakoids from stressed

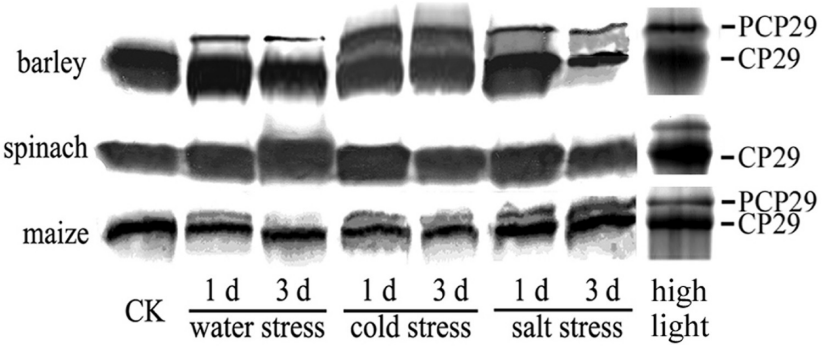

FIGURE 2: Difference in CP29 phosphorylation in barley, maize, and spinach under water, cold, or salt stress or high light. Leaf samples were collected after four different treatments and frozen in liquid nitrogen. Thylakoid membranes were isolated, and the extent of protein phosphorylation was determined by using a CP29-specific antibody. Gel lanes were loaded on an equal protein basis (20 μg). CK represents control seedlings.

Table 1: Values of $1 - q_P$ for Barley Seedlings Exposed to Different Environmental Stresses[a]

control	high light	water stress for 1 day	water stress for 3 days	salt stress for 1 day	salt stress for 3 days	cold stress for one day	cold stress for 3 days
0.11 ± 0.2	0.41 ± 0.4	0.13 ± 0.2	0.36 ± 0.3	0.12 ± 0.2	0.28 ± 0.3	0.24 ± 0.3	0.32 ± 0.4

[a]Plants grown with 100 μmol of photons m^{-2} s^{-1} at 20 °C were subjected to water stress (−0.5 MPa), salt stress (0.3 mol of NaCl/L), or cold stress (4 °C) for 1 day or 3 days or high-light stress (1000 μmol of photons m^{-2} s^{-1}) for 2 h. Mean values for five plants (±standard deviation) are shown.

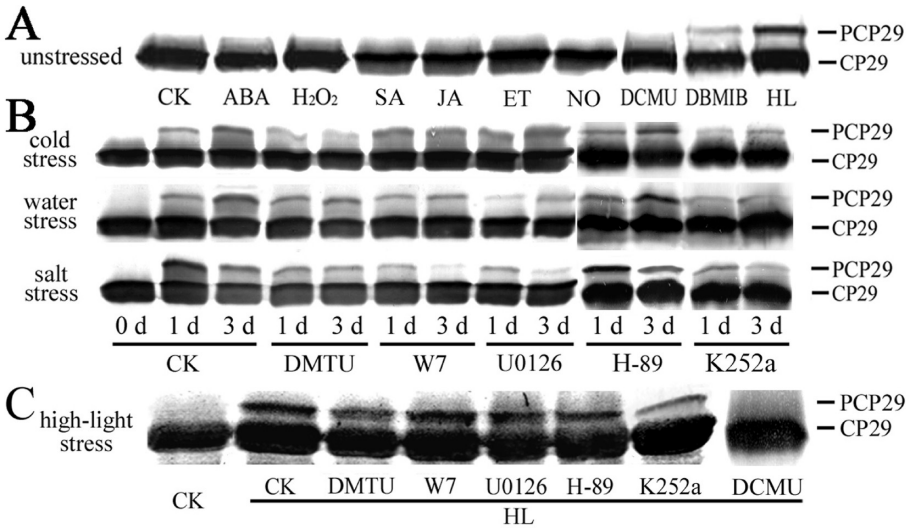

FIGURE 3: Effects of chemicals on CP29 phosphorylation in vivo. (A) Barley seedlings were treated with 100 μM ABA, 10 mM H_2O_2, 0.2 mM SA, 100 μM JA, 150 mg/L ethephon (ET), 0.5 mM SNP (a donor of NO), 20 μM DCMU, or 20 μM DBMIB for 24 h or high light (1000 μmol of photons m^{-2} s^{-1}) for 2 h. Then thylakoid proteins of plants were separated by SDS−PAGE and probed with a CP29-specific antibody. (B and C) Plants were pretreated with 100 μM W7, 25 μM U0126, 50 μM H-89, 20 μM K252a, or 5 mM DMTU and then exposed to cold, water, or salt stress for 1−3 days (B) or high-light stress for 2 h (C) in the presence of W7, U0126, H-89, K252a, or DMTU. Samples were analyzed by immunoblotting. Gel lanes were loaded on an equal protein basis (20 μg). CK denotes control seedlings.

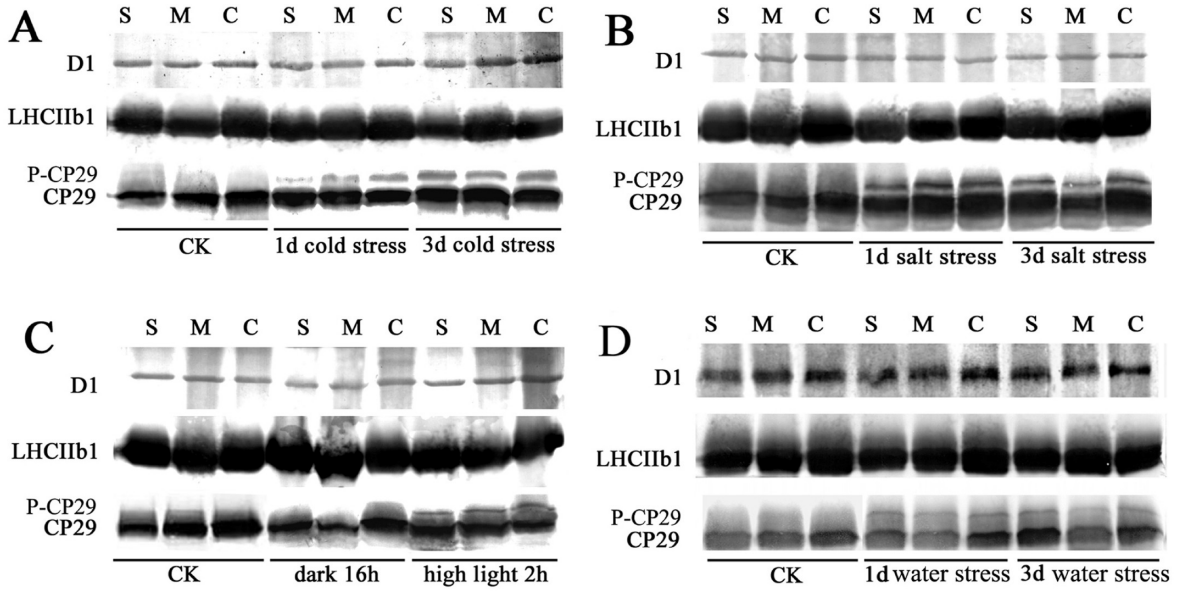

FIGURE 4: Distribution of barley PSII protein complexes under different stresses. Thylakoid membranes from different treatments were subfractionated into grana core (C), grana margins (M), and stroma-exposed (S) regions and analyzed by SDS−PAGE. D1, LHCIIb1, and CP29 were detected using specific antibodies. Gel lanes were loaded on an equal protein basis (20 μg). CK denotes control seedlings. (A) Cold stress. (B) Salt stress. (C) Dark adaption for 16 h and subsequent high-light stress for 2 h. (D) Water stress.

plants into grana lamellae, grana margin, and stroma lamellae. Our previous research suggested that D1 and LHCIIb1 do not laterally migrate from grana to stroma lamellae, but CP29 laterally migrates to the stroma lamellae under water stress (*15*). Here we show similar distributions and migrations under cold stress, salt stress, or high-light stress (Figure 4 and Figure S2 of the Supporting Information). It seems that lateral migration of CP29 is a widespread phenomenon in barley as a response to environmental stresses. Phosphorylation of CP29 necessarily results in its lateral migration.

Disassembly of PSII Complexes and LHCII Trimers under Environmental Stresses. To investigate effects of the environment stresses on the structure of protein complexes, we solubilized the PS complexes from thylakoid membranes using DM and separated them by BN-PAGE. Six major pigment−protein complexes were revealed (Figure 5), representing PSII−LHCII supercomplexes (band I), monomeric PSI and dimeric PSII (band II), PSI and LHCI (band III), monomeric PSII (band IV), trimeric (band V), and monomeric LHCII (band VI) (*28*). The BN-PAGE and immunoblotting analysis clearly indicated that the amount of PSII supercomplexes from control seedlings was larger than that under the stressed conditions. Moreover, the level of LHCII trimers was also decreased under environmental stresses, especially under the severe stresses (for 72 h) (Figure 5 and Figure S3 of the Supporting Information).

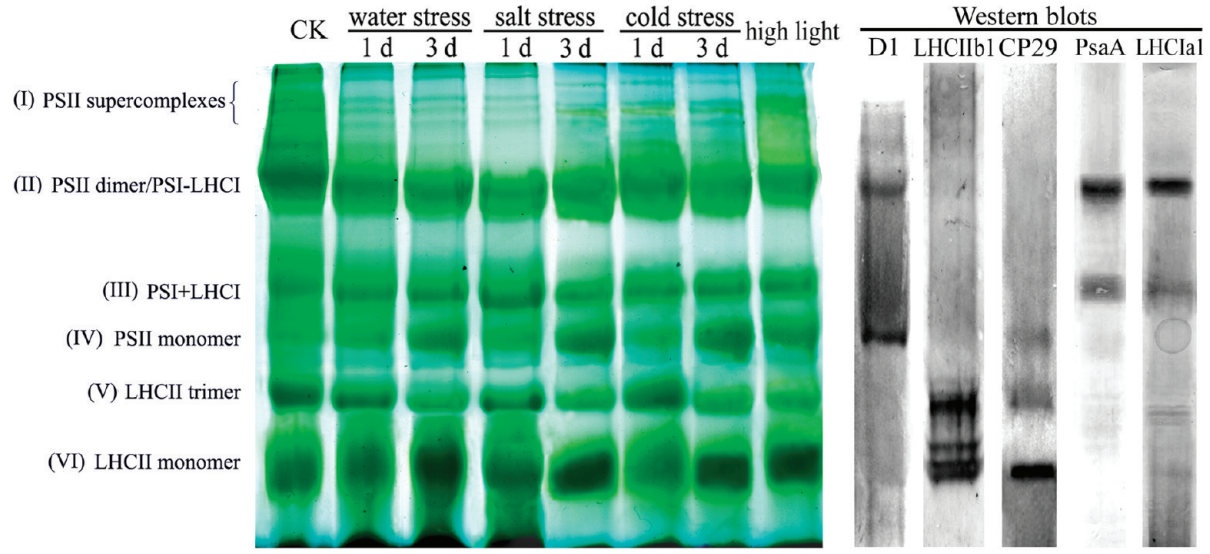

FIGURE 5: Blue native gel analysis of photosynthetic pigment−protein complexes from barley. Thylakoid membranes were solubilized with 1% DM and separated by BN-PAGE. The positions of protein complexes were marked according to Ciambella et al. (*28*). Gel lanes were loaded on an equal Chl basis (60 μg). The bands were confirmed by immunoblotting with D1, LHCIIb1, CP29, PsaA, and LHCIa1 specific antibodies (black and white lines). The control line of the BN gel was selected for immunoblotting.

DISCUSSION

Difference in CP29 Phosphorylation between Monocots and Dicots. Previous research indicates that the chlorophyll *a*/*b*-binding protein CP29 undergoes phosphorylation in C3 (barley) and C4 (maize) under cold stress (*5*, *29*), and *Arabidopsis* also shows CP29 phosphorylation in the PSII light (*7*). However, there is little evidence of CP29 phosphorylation under other environmental stresses. In this paper, we show that CP29 could be phosphorylated in monocotyledonous plants under water, salt, or cold stress, but not in dicotyledonous plants. However, high light induces CP29 phosphorylation in both monocotyledonous plants and dicotyledonous plants. From Table 1, one could notice that $1 - q_P$ reached > 0.4 after high-light stress for 2 h. We infer that there is a threshold to $1 - q_P$ for CP29 phosphorylation, and the thresholds to dicotyledonous plants might be much higher than those for the monocotyledonous plants. Therefore, spinach CP phosphorylation occurs only under strong reduction of the plastoquinone pool (such as high-light stress), but not upon modest plastoquinone reduction (such as cold stress).

Mechanistic Analysis of CP29 Phosphorylation. The rapid CP29 phosphorylation under slight water or salt stress does not depend on the redox state of the plastoquinone pool. What is the regulatory mechanism for this process? In *Arabidopsis*, it is well-known that CP29 phosphorylation is under the control of a plastid protein kinase STN7, which is a plastid Ser/Thr protein kinase, responsible for plastoquinone redox changes (*7*−*12*). Our results suggest that MAPK and CDPK are also involved in the rapid CP29 phosphorylation in barley under slight water or salt stress, which is independent of the plastoquinone pool. We rule out the possibility that MAPK or CDPK functions upstream of STN7 and then acts on CP29 phosphorylation, because of the fact that the Ser/Thr protein kinase inhibitor K252a could not inhibit CP29 phosphorylation entirely under slight stresses (Figure 3B,C and Figure S1 of the Supporting Information). Moreover, upon plastoquinone overreduction (such as high-light or cold stress), K252a did not inhibit CP29 phosphorylation completely either. This is not due to the low inhibition efficiency or the low concentration of K252a we used. Barley STN7 protein kinase activity was completely inhibited by 20 μM K252a (data not shown). MAPK, CDPK, and STN7 may work independently on CP29 phosphorylation in barley.

The phosphorylatable threonine residue of CP29 has a CK2 site, which is recognized as a target for CDPK (*30*). Therefore, its phosphorylation is possibly related to some Ca^{2+}-dependent protein kinases. CDPKs are distributed widely in the whole cell, and they may associate with chloroplasts or likely work on plasma membranes (*31*, *32*). However, no evidence shows that any MAPK lies in chloroplasts, and they are usually distributed in nucleus (*31*, *33*). How MAPK influences CP29 phosphorylation in plastids is still a question. We could only assume that MAPK induces CP29 phosphorylation indirectly through some protein kinase cascade.

Significance of CP29 Phosphorylation under Stresses. PSII core protein phosphorylation (especially the phosphorylation of the D1 protein) has a putative role in the PSII repair cycle and maintenance of PSII dimeric structure, which has been demonstrated in vitro and in vivo (*14*, *34*). Phosphorylated D1 protein should migrate to the stroma lamellae in the case of high light. However, our data (Figure 4C) showed that D1 protein experienced no lateral migration under high-light stress in barley. Our previous research suggested that, in barley under water stress, phosphorylation and lateral migration from granum membranes to stroma-exposed lamellae happen only to CP29, and the other PSII proteins may not migrate but be degraded directly in situ (*15*). Here we suggest that this phenomenon may widely exist in monocotyledonous plants. No migration and in situ degradation occur even under high-light stress in barley.

CP29 phosphorylation has been proposed to be involved in dissipation of excess excitation energy and providing protection against photoinhibition (*5*, *29*). It was also suggested that CP29 phosphorylation might be important to state transitions in *Arabidopsis* (*7*) and *Chlamydomonas* (*6*). Besides, phosphorylation of CP29 may induce a conformational change that modifies the chlorophyll organization and lead to nonradiative energy dissipation (*35*). Andersson et al. (*36*) found that the CP29 antisense lines have a decreased level of CP24 protein and

weakened photosystem II function in *Arabidopsis* but were capable of the qE type of nonphotochemical quenching (NPQ).

In this work, we observed distinct changes in the organization and stability of PSII complexes in barley under environmental stresses. The reduction of PSII supercomplexes under environmental stresses could probably be a consequence of the lateral migration and CP29 phosphorylation. CP29 is close to CP47 and CP24 in the PSII−LHCII supercomplex (*37*, *38*). In the absence of CP29, this supercomplex could not be detected and the bound LHCII trimers are released (*38*). Furthermore, studies with a cleavable cross-linking agent revealed the interaction of TSP9 with both major and minor LHCII proteins. These cross-linked complexes contain TSP9 and some other peripheral PSII subunits, CP29, CP26, and PsbS, which form the interface between LHCIIs and the PSII core (*25*, *39*). Therefore, the lateral migration to the stroma lamellae and phosphorylation of CP29 may change the organization of C_2S_2 (the PSII supercomplex containing a dimeric PSII core and two strongly bound trimeric LHCII complexes) and the interaction between TSP9/PsbS and the PSII core (*39*, *40*), consequently resulting in LHCIIs partly dissociating from PSII under the stressed conditions. CP29 phosphorylation in thylakoid membrane may facilitate disassembly of PSII complexes and LHCII trimers and their subsequent degradation and repair cycle of PSII proteins under different stresses.

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SUPPORTING INFORMATION AVAILABLE

Three figures showing the quantitative data for CP29 phosphorylation, migration, and disassembly of PSII complexes and LHCII trimers under different treatments. This material is available free of charge via the Internet at http://pubs.acs.org.

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