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A Post-translational Modification of the Photosystem II Subunit CP29 Protects Maize from Cold Stress*

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The resistance of maize plants to cold stress has been associated with the appearance of a new chlorophyll *a/b* binding protein in the thylakoid membrane following chilling treatment in the light. The cold-induced protein has been isolated, characterized by amino acid sequencing, and pulse labeled with radioactive precursors, showing that it is the product of post-translational modification by phosphorylation of the minor chlorophyll *a/b* protein CP29 rather than the product of a cold-regulated gene or an unprocessed CP29 precursor. We show here that the CP29 kinase activity displays unique characteristics differing from previously described thylakoid kinases and is regulated by the redox state of a quinonic site. Finally, we show that maize plants unable to perform phosphorylation have enhanced sensitivity to cold-induced photoinhibition.

In the photosynthetic apparatus of higher plants, the efficiency of the electron transport and light-harvesting functions is regulated in response to environmental and metabolic conditions. The earliest described mechanism is the state I-state II transition and consists of the phosphorylation-induced redistribution of light-harvesting complex II (LHCII)[1] (1) and cytochrome b_6/f (2) complexes between grana- and stroma-exposed membranes, thus yielding a modulation of cyclic *versus* linear photophosphorylation (2). A second mechanism has been described for the thermal dissipation of the excitation energy excess. This process is dependent on the deepoxidation of the xanthophyll violaxanthin to zeaxanthin (3), which is mainly located in the minor photosystem II (PSII) subunits CP26 and CP24 (4, 5).

Recently, a new regulatory process has been reported that is elicited by strongly photoinhibitory conditions such as those produced by illumination of maize plants at low temperature. Under these conditions, a new thylakoid protein appears in cold-resistant cultivars and is absent from cold-sensitive plants. While the protein has been isolated and characterized as a chlorophyll a/b protein located close to the PSII reaction center (RC), its origin is still unclear.[2] In particular, it is not known whether the cold-induced SDS-PAGE band is the product of a previously silent *lhcb* gene or a post-translational modification of an existing chlorophyll protein such as CP29, with which it shares epitopes (6, 7) and pigment composition.[2] In this study, we have characterized the cold-induced protein by amino acid sequencing and pulse-chase with radioactive precursors, showing that it is the result of CP29 phosphorylation. Moreover, we report that this phosphorylation induces a change in the CP29 conformation and that the kinase activity is controlled by a quinonic site closely associated to photosystem II RC. Finally, we show that maize plants unable to perform CP29 phosphorylation are more sensitive to photoinhibition.

This is the first report of CP29 phosphorylation and suggests that the reversible modification of membrane-intrinsic, chlorophyll a/b binding proteins can be a mechanism for the modulation of light harvesting and excitation energy transfer to the reaction center.

EXPERIMENTAL PROCEDURES

Preparation of Thylakoid Membranes—*Zea mays* seedlings (cvs. *Adon*, *DK300*, *Oh7N*, and *H93*, supplied by Dekalb Co., IL) were grown for 2 weeks in a growth chamber at 28/21 °C day/night at a light intensity of 200 μE m^{-2} s^{-1} and 80% humidity. Leaves from 2-week-old plants were harvested at the end of a 6-h illumination period at 25 or 4 °C, and thylakoids from mesophyll chloroplasts were prepared as previously described (8). PSII membranes (BBY particles) were obtained according to the method of Berthold *et al.* (9) with the modification described in Ref. 10. Aliquots were suspended in 50 mM Hepes/KOH, pH 7.5, 5 mM $MgCl_2$, 50% glycerol and frozen at −80 °C until required. Membrane yield was determined by measuring chlorophyll content in 80% acetone, using the equations of Porra *et al.* (11).

PAGE and Immunoblotting—For the purification of CP29 and CP34 apoproteins, two consecutive preparative SDS-PAGE were used to avoid minor contaminations. The first elecrophoresis was run on a 12–18% polyacrylamide gradient gel containing 6 M urea with the Tris/sulfate buffer system, as previously described (8). Bands were excised and rerun on a second gel of uniform polyacrylamide concentration (10%) without urea in the Tris/tricine buffer (12). Two-dimensional electrophoresis was performed using the non-denaturing Deriphat-PAGE (on a 5–12% polyacrylamide gradient gel) (13) for the first dimension and the SDS-urea-PAGE (9–16% gradient gel) according to Laemmli (14), with double buffer concentration for the second dimension. Individual spots in the two-dimensional gels were identified by immunoblotting with specific antibodies as previously described (13). One-dimensional electrophoresis was always performed by the Tris/sulfate, 6 M urea system mentioned above. For immunoblot assays, samples were separated by PAGE and electro-transferred to nitrocellulose filters (Hoefer). Filters were then probed with α-CP29 antibodies, and antibody binding was detected by alkaline phosphatase-conjugated α-rabbit IgG (Boehringer Mannheim). Antibodies were raised in rabbits, using poly(A)·poly(U) as adjuvant, and characterized as previously described (15).

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[1] The abbreviations used are: LHCII, light-harvesting complex II; PSII, photosystem II; PSI, photosystem I; RC, reaction center; DM, dodecyl maltoside; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; HPLC, high pressure liquid chromatography; μE, microeinstein; PAGE, polyacrylamide gel electrophoresis.

[2] Mauro, S., Dainese, P., and Bassi, R., submitted for publication.