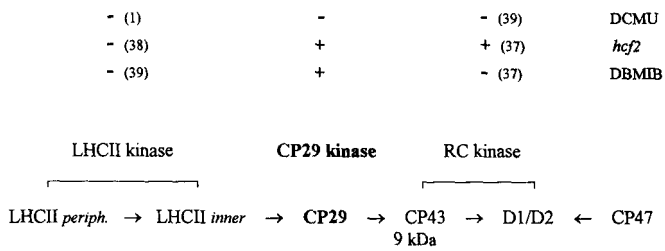

FIG. 8. **Phosphorylation of the components of photosystem II.** Scheme redrawn from Allen (32) is shown; *arrows* show the direction of energy transfer. In the *upper part* of the figure, signs + and −, respectively, indicate activation and inactivation of the kinases by DCMU, DBMIB, and in the cytochrome b_6/f-less mutant (*hcf2*).

three thylakoid protein kinases.

Where is the CP29 kinase located? It has been reported that the 64-kDa LHCII kinase is localized in grana domains, particularly at the edges of the grana stacks, where it is associated to the cytochrome b_6/f complex, which modulates its activity (34). In the mutant lacking this complex, we noticed that the CP29 kinase is constitutively activated, but it does retain its redox sensitivity since DCMU, *i.e.* oxidation of plastoquinol to plastoquinone, turns it off.

The redox sensitivity, the inactivation by DCMU, and the activation by DBMIB indicate that the CP29 kinase must be associated with at least one quinonic site. On one hand, results obtained with DCMU both in wild type and *hcf2* clearly show that the kinase senses the electronic state of the Q_B site of PSII. On the other hand, the activation by DBMIB suggests a negative control played by the Q_Z site of the cytochrome b_6/f, to which DBMIB binds in wild type (43), displacing the quinone on the cytochrome complex and thus mimicking the *hcf2* condition. We propose as a working hypothesis that the CP29 protein kinase is located in grana partitions, in between PSII (where it is in contact with the Q_B site) and the cytochrome b_6/f complex (in contact with the Q_Z site). This would also mean an intermediate position between the RC kinase and the LHCII kinase, respectively. If we consider the models so far proposed for the topological organization of photosystem II (21, 44), our hypothesis is consistent with the position of CP29 between the RC and LHCII, which are substrates of phosphorylation by the different kinase (Fig. 8). An integrated scheme for the redox control of thylakoid protein phosphorylation cannot be drawn as yet and is beyond the aim of this work.

Concomitant with the phosphorylation of CP29, cold treatment induces phosphorylation of the RC subunits CP43, D1, and D2 and 9 kDa in the monomeric form of PSII. Since this corresponds to a dissociation of RC dimers into monomers, it can be hypothesized that this phosphorylation event might be the cause for dissociation of PSII dimers. Phosphorylation-induced dissociation of neighboring chlorophyll-proteins has been previously described (45); similarly, both PSII RC subunits and CP29 phosphorylation can be related to a regulatory mechanism based on the dissociation of the PSII supermolecular complex. These events could be part of at least two regulation phenomena, the first being the dissociation of antenna complexes from PSII and their migration into the stroma membranes (1, 45, 46), with consequent reduction of PSII antenna size, and the second being PSII cycling whose primary step could well be the RC dimer dissociation (47).

Free lateral migration of LHCII and PSII RC subpopulations from the grana stacks and their margins to stroma-exposed thylakoid membrane is an obvious requirement for such mechanisms. Thylakoid membrane fluidity is strongly decreased at temperatures lower than 10 °C (48); we can therefore argue that, under conditions chosen for our experiments (4 °C), the phosphorylated RC subunits of monomeric PSII accumulate since subsequent steps in their cycling are impaired.

Which is the role of CP29 phosphorylation? We approached this question by using two maize lines phosphorylating CP29 to a different extent and evaluating their resistance to photoinhibition following treatment in cold and light. The results showed that the line more active in CP29 phosphorylation is also more resistant to photoinhibition. In fact, although CP29 is not the only protein that is phosphorylated in the cold, PSII core complex subunits and LHCII polypeptides are phosphorylated both in resistant and sensitive lines. This is in agreement with previous data obtained by the analysis of two lines sharing the same genetic background but differing in the level of CP34 induction.[2] In that study, it was also shown that the protective effect was located in the antenna system rather than in the RC complex and consisted in a decreased efficiency in the energy transfer from the antennas to the RC. CP29 phosphorylation isolates an important portion (30%) of PSII antennas, which are connected to the RC through CP29 (32, 45), and may influence chlorophyll and/or carotenoid organization within the complex, thus leading to a decreased energy transfer to PSII RC. While the disconnection of PSII chlorophyll proteins seems to be a general consequence of photoinibitory conditions (47) irrespective of CP29 phosphorylation, spectral differences were actually detected between CP29 and CP34,[2] with a decrease in the absorption of the Chla spectral forms, which are more effective in the energy transfer to PSII RC. This hypothesis is consistent with the observed increase in F_o during the treatment in the cold (18).

We therefore propose that CP29 phosphorylation is a novel photoprotection mechanism, based on the reorganization of pigments in the chlorophyll-protein complex, in such a way that a lower amount of excitation energy is funneled to RCII. Further experiments are in progress to obtain a better characterization of the spectral and structural changes induced by CP29 phosphorylation.

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