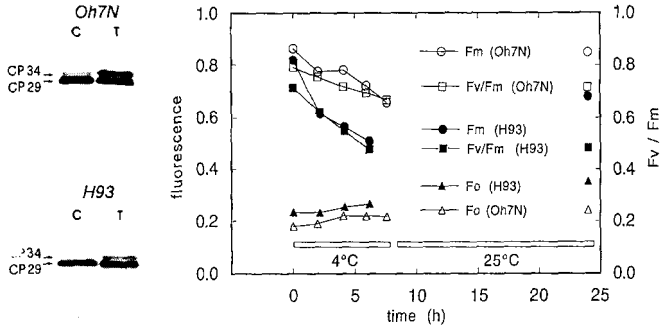

FIG. 7. **Decrease of the photosynthetic quantum yield caused by chill photoinhibition and subsequent recovery.** Two maize lines showing different levels of cold-induced phosphorylation (immunoblot detection of CP29 and CP34 in control (*C*) and cold-treated membranes (*T*) is shown on the *left*) were illuminated in the cold, and fluorescence parameters were recorded over the period of photoinhibitory treatment at 4 °C and at the end of the following recovery, 15 h at 25 °C.

lines were screened by exposing seedlings to the photoinhibitory treatment, followed by SDS-PAGE and immunoblot analysis (not shown) or fluorescence analysis. For example, the two lines *H93* and *Oh7N* differed substantially in the level of CP34 after the cold treatment. In *H93*, CP34 represented 9% of the sum (CP29 + CP34), while in *Oh7N* CP34 represented 33%. As shown in Fig. 7, under high light (1000 $\mu E^{-2} s^{-1}$) at 4 °C, the F_v/F_m fluorescence ratio decreased rapidly in the line *H93* (from 0.75 to 0.48 in 6 h), mainly due to a rapid decrease of F_m. The decrease in F_v/F_m was much smaller in *Oh7N*, from 0.79 to only 0.68, even after 8 h of treatment. This shows that the photochemistry of PSII in the line *Oh7N* was much better protected under stress conditions than in the line *H93*. The recovery of the F_v/F_m ratio after 15 h at room temperature confirms that the PSII reaction centers were more irreversibly photoinhibited at 4 °C in *H93* than *Oh7N*. Moreover, the resistant line *Oh7N* has a capacity to recover from the stress, which is almost absent in *H93*. This overall sensitivity of the photochemistry to cold stress was observed in several lines and was correlated with their ability to phosphorylate CP29, suggesting that this modification of the protein could be involved in a photoprotective mechanism. Although fluorescence results must be cautiously compared between different leaves, we are confident that the differences in fluorescence induction values of the two maize lines are significant. In fact, the same figure was obtained for 14-, 18-, and 21-day-old plants while, for each age, chlorophyll content and deepoxidation state of xanthophyll at the end of the treatment showed very small differences, if any (within 3 and 2%, respectively).

DISCUSSION

The aim of this work was to characterize the nature of the 34-kDa (CP34) SDS-PAGE band, which appears in maize thylakoid membranes following exposure to cold in the presence of light. This cold-induced polypeptide had been shown to be immunologically related to the chlorophyll *a/b* binding protein CP29, one of the minor antenna complexes of PSII (7). Moreover, Mauro *et al.*[2] isolated the two complexes in their native forms and showed that the spectral properties and the pigment composition were very similar. We further characterized CP29 and CP34 by amino acid sequencing, showing that the two polypeptides, homologous to the barley CP29, were identical over the 32- and 20-amino acid-long stretches determined. This strongly suggested that CP34 was not the product of a different gene but rather of an alternative splicing of the *lhcb4* messenger, the precursor of CP29, or a post-translational modification of it.

The ^{35}S labeling experiment discriminated between these hypotheses since the synthesis of CP34 was not enhanced, as would be the case if it was either the product of alternative splicing or the precursor complex. We thus concluded that CP34 is a post-translationally modified form of CP29 and proceeded to the identification of the nature of the modification. This was made difficult by the fact that only *in vivo* treatment is effective in inducing CP34 accumulation while treatment of isolated thylakoids is not. As suggested by the rapid formation and degradation of the protein, we succeeded in labeling CP34 by feeding $H_3^{33}PO_4$ to the plant prior to treatment in the light. Not only was CP34, in contrast to CP29, heavily labeled, but the treatment of the membranes with alkaline phosphatase restored CP29 mobility, thus confirming that the post-translational modification of CP29 was a phosphorylation.

Alkaline phosphatase treatment of thylakoids and PSII membranes, which, respectively, expose stromal and lumenal faces, showed that the phosphorylation site is located on the stromal side of the membrane. This post-translational modification is effective in modifying the protein structure as judged by the limited proteolysis experiment (Fig. 3) and by the change in SDS-PAGE mobility, similar to that described for other phosphoproteins (30). We concluded that the SDS-PAGE band induced by the light treatment in the cold is the phosphorylation product of the chlorophyll *a/b* protein CP29.

Regulation of function of thylakoid proteins by phosphorylation is a well known mechanism that drives lateral migration of LHCII antenna complexes in state I-state II transitions. LHCII kinase has been shown to be activated by plastoquinol and inactivated by plastoquinone; the role of plastoquinol has been documented in the phosphorylation of all other thylakoid proteins, but authors are far from an agreement on the question of how many kinases exist in thylakoids (for a review, see Refs. 31 and 32). The redox sensitivity of the LHCII kinase is provided *in vivo* (37–39), as well as *in vitro* (34), by its association with the cytochrome b_6/f complex.

We tested if the same kinase was also responsible for the phosphorylation of CP29, another member of the *CAB* family. In addition to a wild type line, we analyzed the maize mutant *hcf2*, which is known to lack the activity of the LHCII kinase as a consequence of the absence of the cytochrome b_6/f complex, while the phosphorylation of the four PSII core proteins is largely unaffected (37, 39). We noticed that the phospho-CP29 (CP34) was constitutively present in the thylakoid membranes of this mutant. A pretreatment with DCMU, which prevents all known thylakoid protein phosphorylation by blocking the reduction of plastoquinone (40), totally abolished phosphorylation of CP29 in both wild type and *hcf2* lines even when chilling treatment was applied. The sensitivity of the CP29 kinase to inhibition by DCMU points to a redox control at the level of plastoquinone, with plastoquinol being the activator of the enzyme. However, in contrast to the LHCII kinase, referred to as the 64-kDa kinase, and likewise the putative RC kinase of PSII core proteins, the activation of the CP29 kinase does not require an active cytochrome b_6/f complex.

On the other hand, PSII core proteins are phosphorylated in isolated thylakoids while CP29 is not (41), thus suggesting that the complexes are not substrates of the same kinase. Finally, preliminary results of experiments with DBMIB revealed that this halogenated quinone analogue clearly enhances phosphorylation of CP29 while it inhibits both LHCII and PSII polypeptide phosphorylation (31, 32, 37, 38, 42). DBMIB induces the CP29 kinase activity in the concentration range from 1 to 100 μM, even in dim light without cold treatment. Taken altogether, our results bring new evidences for the existence of at least