Conformational Changes Induced by Phosphorylation in the CP29 Subunit of Photosystem II[†,‡]

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ABSTRACT: Light energy absorbed by the chloroplast membranes of higher plants is dissipated by nonradiative de-excitation in order to protect against photodamage. In photosystem II, which is the photosynthetic component most sensitive to photoinhibition, three pigment binding subunits, called CP24, CP26, and CP29, have been proposed to act in the regulation of the chlorophyll excited states concentration. In heavy stress conditions, CP29 becomes phosphorylated in its stroma-exposed portion, and this process is reversed by returning to normal conditions. In this study, we have used the pigments bound to the intramembrane hydrophobic portion of the protein as intrinsic probes to detect conformational changes induced by phosphorylation. We isolated the phosphorylated and unphosphorylated forms of the protein and showed that, although they have the same pigment complement, spectral differences can be consistently detected by absorption, linear dichroism, and circular dichroism spectroscopy. Alkaline phosphatase treatment of the phosphoprotein restores both the electrophoretic mobility and the spectral properties of the unphosphorylated CP29. The results of this study show that phosphorylation of CP29 can modulate the spectral properties of this photosystem II subunit and provide a possible mechanism for the regulation of excitation energy supply to the reaction center.

Reversible phosphorylation is one of the most efficient mechanisms for the regulation of protein function (Johnson & Barford, 1993). In a few cases, the changes in protein structure, underlying regulation, induced by phosphorylation, are known from X-ray analysis: addition of charged phosphate groups to isocitrate dehydrogenase has been shown to modify the local charge distribution around the active site. The substrate binding is prevented due to charge repulsion, thus obtaining inactivation (Barford et al., 1991). Alternatively, phosphorylation may occur in a protein region located far away from the active site, and function is then regulated through a long-range conformational change involving secondary and tertiary structures as shown in the case of glycogen phosphorylase (Sprang et al., 1988).

In the chloroplast membrane, reversible phosphorylation occurs in a number of photosystem (PS)[1] II subunits [reviewed by Allen (1992)]. Phosphorylation of light harvesting chlorophyll−protein (LHCII) is involved in a mechanism of excitation energy distribution between PSI and PSII: in its unphosphorylated form, LHCII is connected to PSII, to which it transfers excitation energy driving electron transport from water to plastoquinone (QB). When PSII electron transport exceeds PSI activity, the resulting quinone reduction activates a LHCII protein kinase. Upon phosphorylation, LHCII dissociates from PSII centers and instead becomes connected to PSI, whose increased electron transport activity reequilibrates the redox state of plastoquinone, thus obtaining optimal distribution of excitation energy between photosystems (Allen et al., 1981). The molecular mechanism of this process has been proposed to involve a local conformational change in the N-terminal region of the LHCII polypeptide, which includes the phosphorylation site (Thr 6) (Allen, 1992).

Reversible phosphorylation of another PSII subunit, called CP29, has been recently reported (Bergantino et al., 1995). This chlorophyll−protein complex is a minor component of the PSII antenna, located in between the major LHCII complex and PSII core, thought to act in the regulation of the chl *a* excited state concentration of PSII because of its ability of sensing lumenal pH (Walters et al., 1994) and binding violaxanthin (Bassi et al., 1993). Phosphorylation occurs in photoinhibitory conditions and is activated by overreduced plastoquinone through a receptor site distinct from the one controlling LHCII kinase (Bergantino et al., 1995).

In this study, we have analyzed the spectral properties of the phosphorylated and unphosphorylated forms of CP29. We show that the two forms differ for the organization of their chlorophyll chromophores and that these differences are reversed by alkaline phosphatase treatment. Since the phosphorylation site is located on the hydrophilic stretch exposed on the stromal side of the membrane (Bergantino et al., 1995) while chlorophylls seat on the intramembrane hydrophobic region (Kuhlbrandt et al., 1994), we suggest

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[1] Abbreviations: CD, circular dichroism; LD, linear dichroism; chl, chlorophyll; EDTA, ethylenediaminetetraacetate; Hepes, *N*-2-(hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; LHCII, light-harvesting complex of PSII; PAGE, polyacrylamide gel electrophoresis; PS, photosystem; SDS, sodium dodecyl sulfate; tricine, *N*-[tris(hydroxymethyl)-methyl]glycine; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

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that CP29 phosphorylation affects its structure through a long-range conformational change rather than through a local conformational change.

The nomenclature of chlorophyll *a/b* proteins may appear somehow confusing. According to a recent work (Jansson et al., 1992), CP29, CP26, and CP24 are respectively the products of *Lhcb4*, *Lhcb5*, and *Lhcb6* genes while the major LHCII complex is a heterotrimeric protein composed of the products of *Lhcb1*, *-2*, and *-3* genes. These proteins have also been called LHCIIa (CP29), LHCIIb (LHCII), LHCIIc (CP26), and LHCIId (CP24) by the group of Thornber (Peter & Thornber, 1991). Throughout this paper, the phosphorylated form of CP29 is indicated as CP34 according to the initial report (Bergantino et al., 1995).

MATERIALS AND METHODS

Preparation of the Thylakoid Membranes. Seedlings of *Zea mays* (cv Dekalb DK300) were grown for 2−3 weeks in a growth chamber at 12/12 h light/dark and 28/21 °C day/night at a light intensity of 200 μE m^{-2} s^{-1} and 80% humidity. Leaves from 2/3-week-old plants were harvested at the end of a 6-h illumination period at 4 °C, and thylakoids from mesophyll chloroplasts were prepared as previously described (Bassi et al., 1985). PSII membranes (BBY particles) were obtained according to the method of Berthold et al. (1981) with the modifications described by Dunahay et al. (1984). Aliquots were suspended in 50 mM Hepes/KOH, pH 7.5, 5 mM $MgCl_2$, and 50% glycerol, frozen in liquid nitrogen, and stored at −80 °C until required. Membrane yield was determined by measuring the chlorophyll content in 80% acetone using the equations of Porra et al. (1989).

Purification of Phosphorylated and Unphosphorylated Forms of CP29. PSII membranes were resuspended in 0.8 M Tris, pH 8.0, in order to remove extrinsic polypeptides, peletted, then washed with 1 mM EDTA, pH 8.0, resuspended in water at 2 mg of chl/mL, and solubilized by adding 2% dodecyl maltoside (DM). The solubilized sample was fractionated by flat-bed isoelectrofocusing as previously described (Dainese et al., 1990). After focusing overnight at 4 °C, the green bands with p*I* between 4 and 5 were harvested and eluted from a small column with 50 mM Hepes−KOH, 0.06% DM, and the green eluate was loaded onto a 12 mL sucrose gradient (0.1−1 M) containing 0.06% DM and 10 mM Hepes, pH 7.6. After spinning for 23 h at 39 000 rpm in an SW41 rotor at 4 °C, the green bands were harvested with a syringe.

SDS−6 M urea−PAGE was performed with the Tris−sulfate buffer system as previously reported (Bassi et al., 1985).

Spectroscopy. LD and absorption spectra at 280 and 4 K were obtained as described by Haworth et al. (1982) using samples oriented by the polyacrylamide gel squeezing technique. The instrumental bandwidth was 2 nm. Alternatively, absorption spectra were recorded at 280 K in a solution containing 10 mM Hepes, pH 7.6, 0.06% DM with a Kontron DW2 spectrophotometer using a 1 nm bandwidth. CD spectra were recorded with a Jasco 600 spectropolarimeter with a bandwith of 1 nm in a 5 mm path length cuvette at 280 K.

In Vitro Dephosphorylation. Following spectroscopic analysis, the phosphoprotein sample was divided into two halves. One was treated by adding 20 units of alkaline phosphatase (type VII-SA, Sigma) for 15 min at 20 °C; the other was used as a control. The completion of the dephosphorylation reaction was checked by analyzing small aliquots by denaturing SDS−PAGE as shown in Figure 6A.

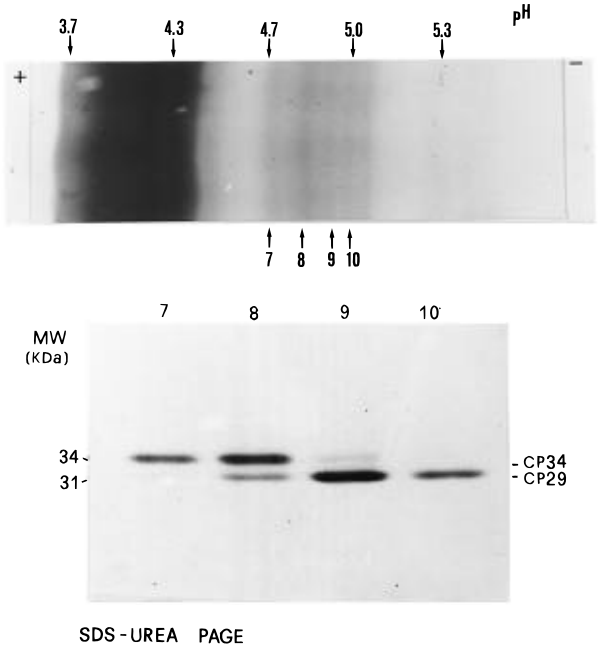

FIGURE 1: Purification of the phosphorylated and unphosphorylated forms of the CP29 subunit of photosystem II. (A) Flat-bed IEF (unstained) of DM-solubilized PSII membranes (BBY particles). pH values (numbers above the gel in the figure) were measured following the end of the run. Green bands containing CP29 and/or CP34 are indicated with numbers from 7 to 10 below the gel. (B) SDS−urea−PAGE analysis: green bands from IEF were submitted to sucrose gradient ultracentrifugation, and the second green band (at 0.3 M sucrose) was harvested with a syringe and aliquots loaded onto the gel. After the run, the gel was stained with Coomassie blue. See Materials and Methods.

Pigment Analysis. Pigments were extracted in 80% acetone. HPLC analysis was performed as previously reported according to Gilmore and Yamamoto (1991).

RESULTS

When PSII membranes, obtained from cold-treated maize plants, were fractionated by flat-bed IEF, the chlorophyll−protein pattern was very similar to that previously described for membranes from control plants (Dainese et al., 1990). Minor differences were evident in the 4.6−4.8 pH range of the IEF gel where CP29 migrates: four green bands were resolved, having p*I* values of respectively 4.68, 4.69, 4.71, and 4.72 (Figure 1A), while only three bands were observed with PSII membranes from control leaves (Dainese et al., 1990). The bands were eluted from the gel and run on a sucrose gradient. The polypeptide composition of the upper band from each sucrose gradient is shown in Figure 1B. The most acidic fraction contained the phosphorylated form of CP29, which was previously called CP34 for its decreased mobility in the SDS−urea gel (Bergantino et al., 1995), with respect to the unphosphorylated form which migrated in the least acidic IEF band. The two intermediate bands contained both CP29 forms in different relative amounts. Densitometric analysis of the four SDS−PAGE lanes of Figure 1B yielded the following relative amounts for the phosphorylated and unphosphorylated forms of CP29: 90/10, 70/30, 9/91,

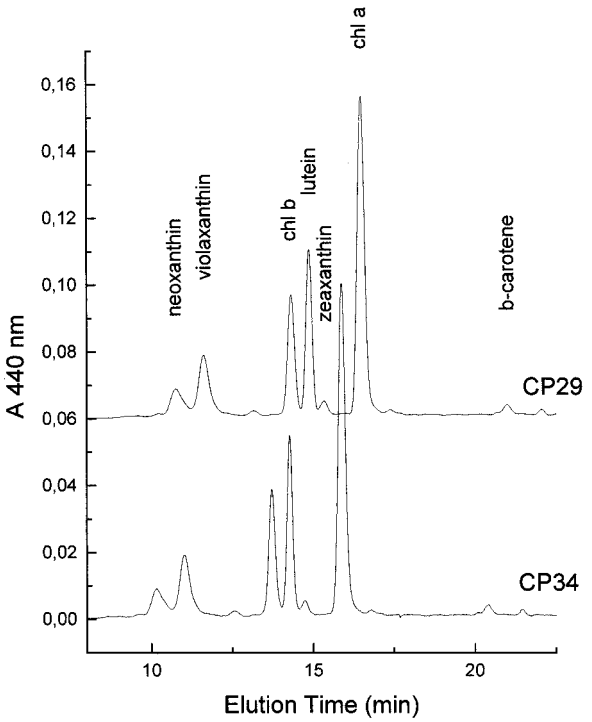

FIGURE 2: Pigment analysis of IEF fractions 7 (CP34) and 10 (CP29). Chlorophyll proteins from sucrose gradient ultracentrifugation were extracted with 80% acetone and analyzed by reverse-phase HPLC as described under Materials and Methods. Detection wavelength was 440 nm. The plot was shifted on the *x* axis in order to avoid superimposition of the major peak. Fractions 8 and 9 (not shown) also had an identical HPLC pattern.

Table 1: Pigment Composition of the Phosphorylated and Unphosphorylated Forms of CP29 As Determined by HPLC Analysis of Acetone Extacts[a]

	chl *b*	lutein	neoxanhin	violaxanthin	zeaxanthin	β-carotene
thylakoids	30	12.2	2.1	1.8	1.2	7.1
CP29	34	19.8	5.9	12	3.8	1.0
CP34	33	20.1	6.1	11.5	4.0	1.1

[a] Values are in moles per 100 mol of chlorophyll *a*. Data are the average of triplicate measurements.

and 3/97. These fractions did not contain contaminations from other thylakoid polypeptides. HPLC analysis of the pigment extracts from the thylakoid membranes of light- and cold-treated plants and from purified CP29 and CP34 is shown in Figure 2 and Table 1: the pigment composition appears to be identical in the two forms of CP29, suggesting that phosphorylation does not affect the stoichiometry of pigment binding to the CP29 polypeptide. Light treatment in the cold induced a substantial conversion of violaxanthin into zeaxanthin (Table 1); however, both CP29 and CP34 bound substantial amounts of violaxanthin but low amounts of zeaxanthin. The violaxanthin to zeaxanthin ratio was identical. It is worth noting that analysis of CP26 and CP24 proteins obtained from the IEF separation of Figure 1 contained much higher amounts of zeaxanthin: up to 8 and 12 mol per 100 chl *a* moles, respectively (not shown).

Phosphorylation of CP29 causes a dramatic change in the electrophoretic mobility of the protein as well as changes in its polypeptide folding as revealed by limited proteolysis (Bergantino et al., 1995). We then proceeded to verify whether these effects are restricted to the stroma-exposed N-terminal domain or may affect the whole CP29 structure including the trans-membrane hydrophobic domains which

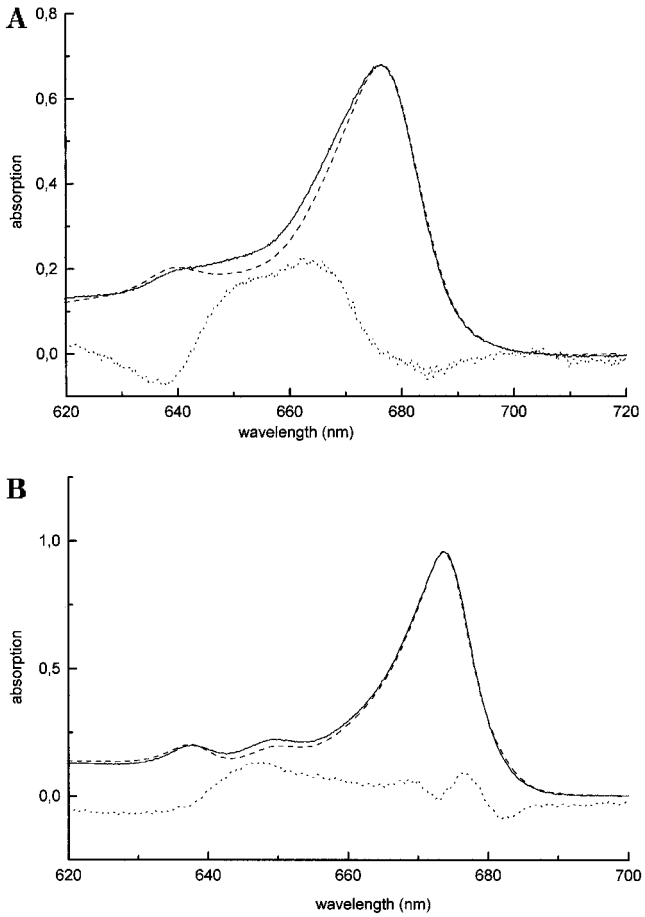

FIGURE 3: Absorption spectra of the unphosphorylated (CP29, fraction 10, dashed line) and the phosphorylated (CP34, fraction 7, solid line) forms of the chlorophyll−protein complex. Lower curve (dotted) represents the phosphorylated minus unphosphorylated difference spectra (×5) after normalization at their maxima. (A) Room temperature (281 K); (B) low temperature (4 K). See Materials and Methods for a detailed description of measurement conditions.

bind chlorophyll and xantophyll molecules with a potential effect on the light harvesting function. According to the available information on the chl *a/b* protein structure (Kuhlbrandt et al., 1994; Bassi et al., 1996), most of the polypeptide is involved in pigment binding, and therefore optical spectroscopy of CP29 allows the use of the pigments as an intrinsic reporter for detection of structural changes induced in these proteins.

Absorption spectra of CP29 and CP34 at RT and 4 K are shown in Figure 3A and Figure 3B, respectively. The spectra of CP29 were identical to those previously reported (Jennings et al., 1993a; Zucchelli et al., 1994). CP34, however, consistently differed in minor features in the 630−670 nm range: at room temperature (RT), the 640 nm small peak of CP29 is only a shoulder in CP34 due to increased absorption at 650−670 nm. This is best seen in the CP34 − CP29 difference spectrum: CP34 was enriched in absorption components peaking at 653 and 663 nm while a 638 nm component was decreased with respect to CP29. At low temperature, the spectra of both CP29 and CP34 were blue-shifted by 3−4 nm compared to RT. Thus, the major positive component in the CP34 − CP29 difference spectrum was evident at 647 nm. An interesting observation is that in the difference spectra, changing the temperature from 298 to 4 K reverses the ratio between the 653 nm and the 663

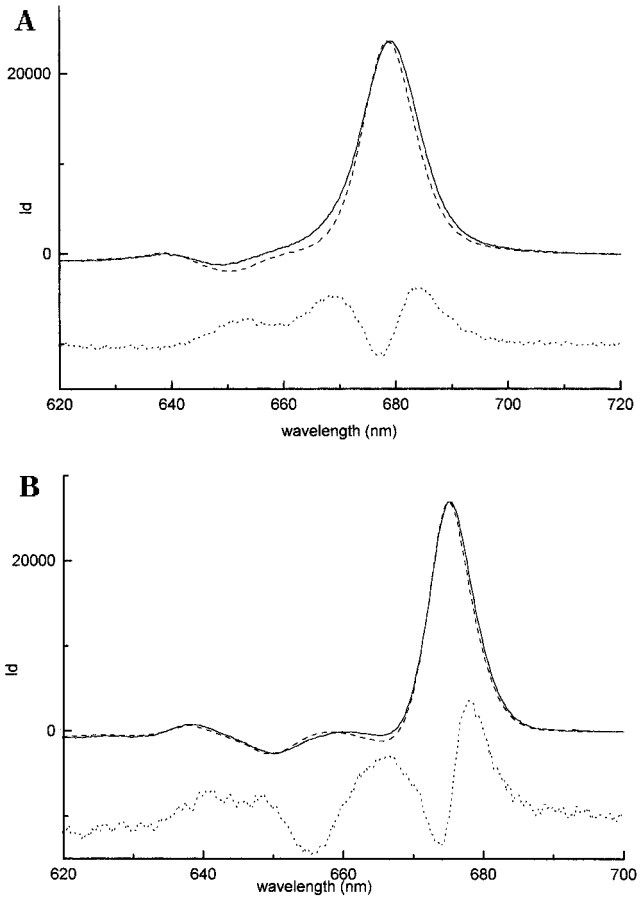

FIGURE 4: Linear dichroism spectra of the same samples analyzed in Figure 3. (A) room temperature (281 K). (B) Low temperature (4 K). See Materials and Methods for a detailed description of measurement conditions.

nm absorptions probably due to differences in the thermal broadening of the chl *a* vs chl *b* absorption components (Zucchelli et al., 1994).

The linear dichroism spectrum at RT of CP29 is shown in Figure 4A and is identical to that previously reported (Zucchelli et al., 1994). The spectrum of CP34 is similar; however, distinctive features are revealed by the CP34 − CP29 difference spectrum showing positive components at 653, 669, and 684 nm. When the spectra were taken at low temperature (Figure 4B), the 653 nm component (RT) was split into two (639 and 649 nm at 4 K) while differences attributable to chl *a* were resolved at 666 and 678 nm due to the blue shift induced by low-temperature conditions.

Circular dichroism analysis (Figure 5) was only performed at room temperature. As previously reported for LHCII (Ide et al., 1987), the spectrum of CP29 was characterized by six major negative components at 680 nm, 645 nm, 638 nm, 498 nm, 474 nm, and 462 nm. When CP34 was analyzed, a shift was shown for the chl *a* signal from 680 to 679 nm, and changes were observed for the signals due to chl *b* absorptions: the 645 nm (−) component is more pronounced than that at 638 nm (−), which is the major one in CP29. In the Soret region, the 474 nm (−) signal becomes dominant over that at 462 nm (−) in CP34 while they have almost the same amplitude in CP29. The 498 nm (−) signal is also blue-shifted by 1 nm. Analysis of IEF fractions 8 and 9, which contain mixtures of CP34 and CP29 in different relative amounts (Figure 5), confirms that the spectral differences are correlated with the extent of phosphorylation in the protein.

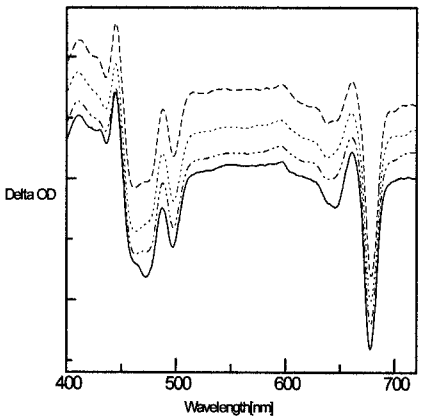

FIGURE 5: Circular dichroism spectra (281 K) of chlorophyll−proteins from IEF fractions 7−10. The upper curve (dashed line) corresponds to the unphosphorylated protein, the lower curve (solid line) to the fully phosphorylated protein. The intermediate curves, from fractions 8 and 9, represent the spectra of mixtures of the phosphorylated and unphosphorylated forms (70% P, fraction 8; 9% P, fraction 9). Principal signals (−) are detected at 462 nm, 474 nm, 498 nm, 638 nm, 645 nm, and 679 nm. See Materials and Methods for a detailed description of measurement conditions.

Table 2: Summary of the Chlorophyll Absorption Forms of CP29 (Zucchelli et al., 1994; This Work) and of the Major Differences Detected following Phosphorylation (This Work)[a]

absorption (RT)	absorption (4 K)	LD (RT)	LD (4 K)	CD (RT)
	CP29			
644.5	638.5	661.0	671.0	680
658.5	650.5	669.0	676.0	645
669	662.5	678.0	681.0	638
677.5	670.0	682.5		498
683	675.0	693.0		474
694.5	679.5			462
	CP34			
638 (−)	638 (−)	653 (+)	666 (+)	679 (←)
653−663 (+)	648 (+)	669 (+)	678 (+)	645 (+)
683 (−)	669 (+)	684 (+)		674 (+)
	675 (+)			
	682 (−)			

[a] All the values are in nm. (+)/(−), increase/decrease of the signal; (←) blue shift.

Although the above described differences between the spectral properties of CP29 and CP34, summarized in Table 2, were consistently observed in at least five independent separations, it was important to obtain positive evidence that these changes were only due to phosphorylation rather than to possible other concomitantly occurring phenomena. This was attempted by studying the reversion of the CP34 spectral differences with respect to CP29 following treatment with alkaline phosphatase (AP).

Figure 6A−C shows the results of such experiments. The AP treatment is fully effective in restoring the CP29 mobility of CP34; concomitantly, also the absorption spectra (Figure 6B) and the CD spectra (Figure 6C) reverse from CP34 to CP29 spectra, thus confirming that the spectral changes described above are only due to CP29 phosphorylation.

DISCUSSION

Small conformational changes induced by phosphorylation have been described for soluble proteins whose crystallographic structures have been elucidated (Sprang et al.,

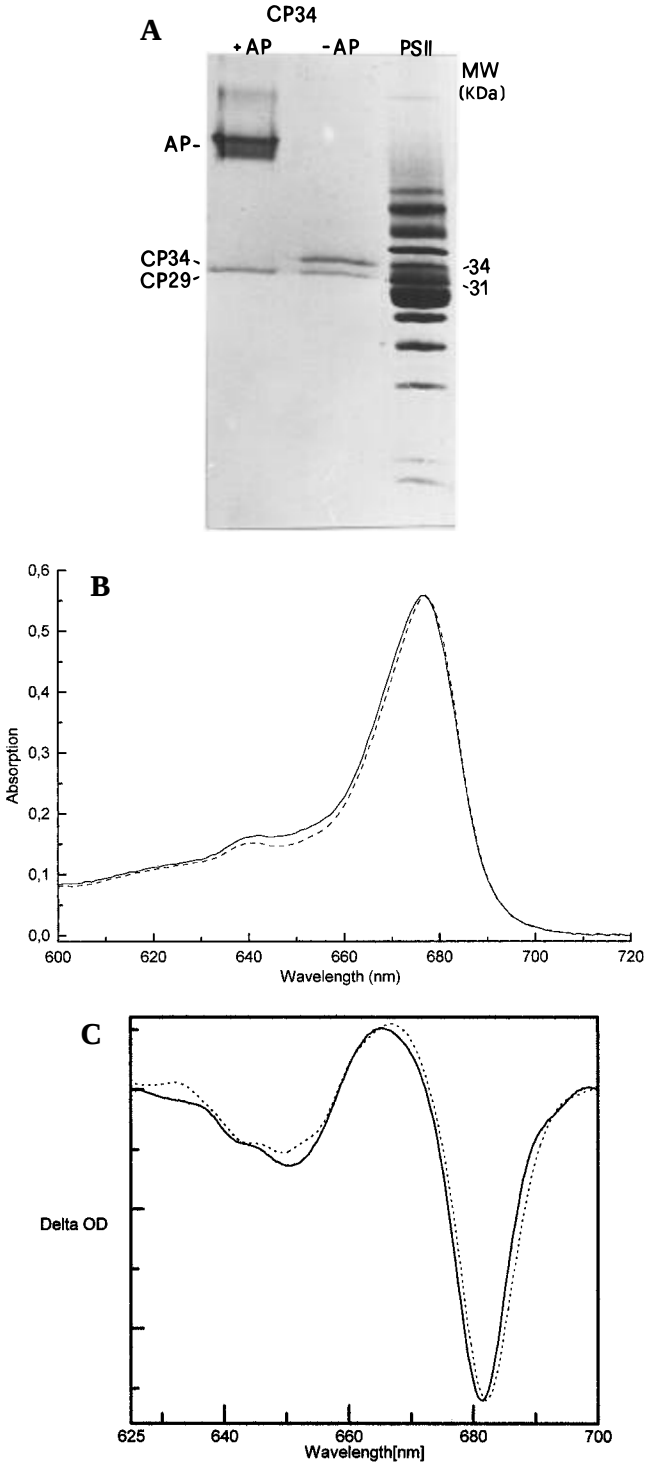

FIGURE 6: Effect of alkaline phosphatase (AP) treatment on the (A) SDS−urea−PAGE polypeptide pattern. PSII, photosystem II membranes. (B) Absorption spectra of phosphorylated (CP34, solid line) and dephosphorylated (CP29, dashed line) forms by AP treatment of the chlorophyll protein. The dephosphorylation treatment was applied by adding the enzyme directly in the cuvette as described under Materials and Methods. (C) CD spectra of phosphorylated (solid line) and dephosphorylated (dotted line) samples of panel B. See Materials and Methods for a detailed description of measurement conditions.

1988; Barfold et al., 1991). A number of membrane proteins undergo reversible phosphorylation, and this process has been proposed to induce important conformational changes which are thought to extend through the membrane, thus altering their function and acting in signal transduction between different cellular compartments as in the case of rhodopsin (Dratz et al., 1983) and γ-aminobutyrate (GABA) receptor (Schofield et al., 1987) or changing the conductivity of water channels in plant seed vacuoles as in the case of aquaporin (Maurel et al., 1995). Nevertheless, experimental evidence for phosphorylation-induced changes in membrane proteins is insufficient due to the difficulty of crystallizing membrane proteins while solution methods such as CD in the far-UV range are unsuitable due to the intrinsic low resolution of the technique (Chang et al., 1978). In this respect, CP29 is particularly suitable due to the binding of six chl *a*, two chl *b*, and two to three xantophyll molecules (Dainese & Bassi, 1991; Bassi et al., 1993) which may be used as internal probes of protein structure by a number of spectroscopic methods.

We have compared the unphosphorylated and the phosphorylated forms of CP29 by absorption, linear dichroism, and circular dichroism spectroscopy. Although the spectra of CP29 and of its phosphorylated form, CP34, are very similar, they consistently differ for a number of features.

Our confidence that these spectroscopic differences can be ascribed to phosphorylation can be summarized by the following: (i) the differences have been consistently detected in the proteins isolated in five independent experiments; (ii) careful analysis by SDS−PAGE and HPLC pigment analysis of the fractions containing phosphorylated and unphosphorylated CP34 confirms the high degree of purity of the samples, and their identical pigment composition thus shows that spectral changes are not due to contamination by other chl binding proteins; (iii) analysis of IEF fractions 8 and 9 (containing both CP34 and CP29) yields spectra with characteristics intermediate between pure CP29 and CP34 according to their relative polypeptide content; (iv) the alkaline phosphatase treatment of CP34 restores the spectral features of CP29 as well as its electrophoretic mobility.

Absorption and circular dichroism spectra consistently indicate that chlorophyll *b* organization is modified by phosphorylation since the major spectral changes are detected in the 635−655 nm range where chl *b* maximally absorbs (Zucchelli et al., 1990, 1992; Jennings et al., 1993b). Moreover, the increased absorption at 660−665 nm indicates that at least a chl *a* absorption form (Jennings et al., 1993b; Zucchelli et al., 1994) is probably affected. Two chlorophyll *b* absorption forms are detected in CP29, respectively absorbing at 638−640 nm and at 648−653 nm (Jennings et al., 1993; Zucchelli et al., 1994). Phosphorylation induces a decreased absorption of the first form while the second is correspondingly increased. It is tempting to attribute these absorption components to the two chl *b* molecules detected in CP29 by biochemical analysis (Dainese & Bassi, 1991; Bassi et al., 1993). The location of chl *b* in the CP29 molecule is not known at present but can be tentatively assigned on the basis of the homology with the light harvesting chlorophyll *a/b* protein of PSII (LHCII) whose structure was resolved at 3.7 Å resolution. In their model, Kuhlbrandt and co-workers (Kuhlbrandt et al., 1994) propose that the chl *a* molecules correspond to the porphyrin rings close to the two carotenoid molecules crossing helices A and B while chl *b* molecules are located more peripherally. Three amino acid residues acting as chl *b* ligands have been tentatively identified in LHCII, two of which are conserved in the CP29 sequence (Morishige & Thornber, 1992; Green & Pichersky, 1993). If the homology with LHCII holds,

then the two chl *b* molecules of CP29 lie close to the stromal side of the membrane, bound to helix C (chl b_5 in the LHCII structure), while the other is bound to the His residue of helix D near the C-terminus and corresponds to chl b_3 in the LHCII model of Kuhlbrandt et al. (1994). Since the His 212 is not conserved in CP24 and yet this complex shows a 640 nm absorption component (Jennings et al., 1993a; Zucchelli et al., 1994), the latter should be attributed to chl b_5 while the 653 nm component could be associated to chl b_3. This is consistent with the higher hydrophobicity which is expected for the chl b_3 site on the basis of the surrounding protein and lipid environment. A tentative explanation for the spectral changes observed for chl *b* is therefore a conformational modification making the chl b_5 environment more hydrophobic.

Linear dichroism spectra of CP34 and CP29 show only small changes in the 630−660 nm range, but this is not surprising since this spectral region is characterized by a low signal and by the presence of vibrational bands of chl *a* absorption forms (Zucchelli et al., 1994). Significant differences between LD spectra of CP29 and CP34 are instead detected above 660 nm, where large signals are observed: major differences were detected at 669 and 684 nm, corresponding to components obtained by Gaussian deconvolution of absorption and LD spectra (Zucchelli et al., 1994). An additional absorption form might be affected, peaking at 677 nm, as judged by the strong shift of the redmost peak in the difference spectrum at 4 K since the 684 nm absorption form is virtually absent at this temperature (Zucchelli et al., 1994). These differences in LD spectra can be compared with the smaller difference in absorption spectra at the respective wavelengths, thus suggesting that they are most probably due to changes in the orientation of the dipole transition (and therefore of the porphyrin ring) with respect to the membrane plane. It should be noted that the small amplitude of the differences in the spectra of the phosphorylated vs unphosphorylated form of CP29 might be due to the normalization of the spectra at the chl *a* peak. However, we consider these small differences as real due to their high reproducibility in the absorption and CD spectra.

Reversible phosphorylation has been shown to be effective in the regulation of excitation energy distribution between PSI and PSII in the photosynthetic membrane (Bonaventura & Myers, 1967). Following phosphorylation on its N-terminus, the major antenna, LHCII, detaches from the PSII core complex and becomes associated with PSI to which it transfers excitation energy. In its molecular recognition hypothesis, Allen (1992) proposed that the decreased affinity of LHCII for PSII and the concomitant association to PSI are caused by conformational changes of the N-terminal part of the protein which is thought to mediate protein−protein interactions on the membrane plane. To the best of our knowledge, no attempt was made to verify if the phosphorylation induces long range effects on LHCII structure, thus involving the pigments bound on its hydrophobic transmembrane region. In the case of CP29, phosphorylation occurs under strong photoinhibitory conditions such as light treatment in the cold or CO_2 depletion (Bergantino et al., 1995), and yet there is no evidence for disconnection of phospho-CP29 from PSII RC. Since maize genotypes exhibiting the enhanced ability to phosphorylate CP29 showed increased resistance to photoinhibition in the cold, phosphorylation may act by modifying the light harvesting and/or transmitting properties of CP29, thus avoiding overexcitation of PSII. Besides its characteristic of being reversibly phosphorylated, CP29 has been previously shown to bind violaxanthin (Bassi et al., 1993), the precursor of zeaxanthin. This xantophyll has been correlated with the process of nonphotochemical quenching (NPQ) of chlorophyll *a* fluorescence, a major photoprotection mechanism in the chloroplast which is elicited by the establishment of a trans-thylakoid pH gradient (Demmig-Adams, 1990; Gilmore & Yamamoto, 1992). Consistently, CP29 has been shown to bind DCCD in a pH-dependent manner (Walters et al., 1994) and to change its fluorescence yield upon aggregation in the presence of zeaxanthin (Ruban et al., 1996), thus suggesting it might be a major regulation site of chlorophyll excited state concentration. In this study, we have shown that the reversible phosphorylation of CP29 in its hydrophilic, stroma-exposed, N-terminal domain affects the organization of chl molecules within the membrane plane, thus implying that long-range allosteric effects are involved. At present, an interpretation of the spectroscopic changes induced by phosphorylation in terms of photoprotection such as increased thermal dissipation of excess chl *a* excited states is not possible due to the low available information on CP29 structure. However, the fact that phosphorylation is elicited by strong photoinhibitory conditions encourages further structural and functional analysis of CP29 in order to verify the hypothesis of its involvement in photoprotection mechanisms.

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