A Protein Kinase in the Core of Photosystem II†

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ABSTRACT: In green plants, several intrinsic protein components of the photosystem II (PS II) complexes are subject to reversible phosphorylation on threonine residues. Evidence from mutant and inhibitor studies indicates that multiple kinases are involved. The protein kinases appear to be membrane-bound and redox-regulated, with activity requiring reducing conditions. We report the identification of a protein kinase activity which copurifies with a core complex of PS II and is capable of phosphorylating the photosystem proteins and associated light-harvesting complex. The enzyme is a distinct and novel protein whose close proximity to the photosystem reaction center is confirmed by its rapid inactivation under strong red light irradiation in the presence of oxygen.

Reversible protein phosphorylation is well established as a mechanism for signal transduction and the regulation of metabolic pathways in both plants and animals. The responsible protein kinases and phosphatases usually show high residue specificity toward defined phosphorylation sites in their protein targets. Phosphorylation of intrinsic protein components of the photosynthetic complexes share some of these characteristics, such as a preponderance of threonine modifications (Bennett, 1991). The modified residues are on the stromal face of the thylakoid membrane, and it is presumed that this membrane is also the site of the kinase-(s) and phosphatase(s) involved in the catalysis of the reactions. Numerous specific enzymes may be required or fewer operating with relaxed substrate specificity. The protein kinase(s) appear to be redox-regulated with activity favored by reducing conditions (Horton et al., 1981). The apparent midpoint potential of activation correlates with the reduction of the membrane plastoquinone (PQ)[1] pool (Horton et al., 1981). The phosphorylation of the thylakoid proteins is thought to bring about an increase in negative charge on the stromal aspect of the membrane under conditions which cause the PQ pool to be over-reduced (Allen et al., 1981). This is postulated to initiate electrostatic detachment of some light-harvesting complex (LHC II) from photosystem II (PS II) with consequent decrease in the electron pressure causing the PQ over-reduction. This negative feedback loop would not be the only means by which PS II is down-regulated, and it is postulated to contribute to balancing the relative turnovers of PS II and photosystem I (PS I), which may be visualized as electron pumps working in series.

Mild, neutral detergents, such as octyl glucopyranoside (OG), have been used to obtain kinase-active extracts from thylakoid membranes (Coughlan & Hind, 1986a; Gal et al., 1990; Race et al., 1995a), and recent studies confirm the involvement of a 64-kDa membrane protein distinct from a polyphenol oxidase of the same mass (Hind et al., 1995; Race et al., 1995b; Sokolenko et al., 1995). Although the bulk of thylakoid kinases is extracted in this way, enzyme activity is consistently detected in the remaining unsolubilized membranes. Such material is likely to retain varying amounts of the four intrinsic thylakoid complexes including PS I and PS II.

In the work described here, established fractionation protocols were employed to identify the origin of the residual membrane-associated kinase activity. This was found to originate in a minor protein component, distinct in apparent mass from the 64-kDa kinase and physically associated with PS II. The apparent tightness of this association prompted a search for evidence of functional interaction of the enzyme with PS II photochemistry. Kinase activity proved to be highly sensitive to illumination by red light in the presence of oxygen. Under conditions favoring photogeneration of oxygen-containing radicals at the reducing side of PS II, and leading to partial photobleaching of photosynthetic pigments, more than 50% of the activity could be destroyed within 1 min. These results lend support to the notion that this novel protein kinase is specifically complexed within PS II. Further studies performed under physiological illumination regimes will be needed to examine whether the redox status of PSII components may effectively regulate the kinase *in vivo*.

EXPERIMENTAL PROCEDURES

PS II-enriched membranes were prepared from spinach as described by Berthold et al. (1981). PS II core complexes and a crude LHC II preparation were derived from this material according to Ghanotakis et al. (1987). Tris-washing of PS II complexes was accomplished as in Ghanotakis et al. (1989). LHC II was further purified by modification of the protocol of Krupa et al. (1987). The crude LHC II remaining after OG solubilization of PS II-enriched membranes was treated with TX-100 [detergent to Chl ratio of 10:1 (w/w)] before precipitation with 100 mM KCl and 10 mM $MgCl_2$. The pellet obtained after centrifugation through a cushion of 0.5 M sucrose (30 000*g*, 40 min) was resus-

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[1] Abbreviations: Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DTT, dithiothreitol; LHC II, chlorophyll *a*/*b*-binding proteins of light-harvesting antenna complex associated with photosystem II; OG, octyl glucopyranoside; PS II−PK, protein kinase closely associated with PS II core complex; PQ, plastoquinone; PS I, photosystem I; PS II, photosystem II; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TX-100, Triton X-100.

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pended in 10 mM Tricine (pH 8.0), 50 mM sorbitol, 1 mM DTT at 0.8 mg of Chl/mL, and LHC II was precipitated with KCl and $MgCl_2$ as above. Centrifugation through sucrose (10 000*g*, 10 min) resulted in a pellet of purified LHC II which was resuspended as before. The OG extract of thylakoids was obtained as outlined in Race et al. (1995a); DTT (1 mM) was included in all buffer solutions. Chl concentrations were determined as described by Arnon (1949), and protein levels were estimated according to Bradford (1976).

Protein kinase activity was measured using a histone III-S phosphorylation assay (Race et al., 1995a): incident illumination during the assay period was minimized ($\leq$ 7 μmol/m^2/s). Casein, phosvitin, histone VIII-S, or myosin light chains (each at 0.5 mg/mL) were substituted in place of histone III-S to assess substrate preference. *In vitro* phosphorylation of PS II proteins and purified LHC II was monitored after 3 min incubation with [γ-^{32}P]ATP (Coughlan & Hind, 1986b). Samples equivalent to 25 μg of protein were analyzed except where PS II and LHC II were mixed, and then the amount of LHC II was reduced to 10 μg to minimize band distortion during electrophoresis. SDS−PAGE was performed using a 13.5% polyacrylamide/4M urea gel (12.5 $\times$ 13 cm; Race & Gounaris, 1993). Incorporation of radioactivity was detected using a Molecular Dynamics PhosphorImager. Non-radioactive, phosphorylated PS II cores were produced in a similar way except that the incubation time was extended to 15 min to ensure maximal modification of the proteins.

The renaturation blot assay used was based on the method of San Agustin and Witman (1995). Samples of OG extract (8 μg of protein), PS II core complexes (8 μg of protein), and catalytic subunit of cAMP-dependent protein kinase (1 μg of protein) were electrophoresed on 10% polyacrylamide minigels. Proteins were transferred to PVDF (Millipore) using 25 mM Tris, 192 mM glycine, 20% (v/v) methanol (140 mA $\times$ 4 h, 4 °C). Renaturation was followed by incubation for 1 h in 30 mM Tris (pH 7.4) with histone III-S (0.5 mg/mL) as indicated. The filters were then treated with [γ-^{32}P]ATP (50 μCi/mL, 6000 Ci/mmol; 15 mL) for 30 min before washing to remove unincorporated label (San Agustin & Witman, 1995). Where indicated filters were incubated with ATP (0.5 mM) before washing. Radioactive proteins were detected as above.

Irradiation of PS II core complexes was performed using red light defined by a Kodak Wratten 25 filter; variable intensities were achieved using mesh screens and metallic neutral density filters. PS II cores (230 μg of Chl/mL) were illuminated at 22 °C in a 1.0 mm pathlength wafer cuvette in contact with a thermostatted copper block; spectra were recorded in the same cuvette. Aliquots were removed at the times indicated in the figure legends, stored on ice in darkness, and assayed within 10 min. When used, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB, 0.2 mM), superoxide dismutase (10 units/μg of Chl), and catalytic subunit of cAMP-dependent protein kinase (0.018 μg of protein/μL, 1.0 unit/μg of protein) were preincubated with the PS II complexes for 5 min in darkness at 22 °C. DTT was excluded when DBMIB was used. Electron transfer kinetics of PS II core complexes were assayed at a concentration of 8.5 μg of Chl/mL in the presence of 1,5-diphenylcarbazide (1.5 mM) and 2,6-dichlorophenolindophenol (50 μM) in 50 mM Tris (pH 7.5), 10 mM NaCl, 5 mM $CaCl_2$ using an SLM DW2000 spectrophotometer. Pigment photobleaching was also monitored with this instrument.

Table 1: Fractionation of Protein Kinase Activities from Spinach Thylakoids[a]

sample	volume (mL)	protein (mg)	chlorophyll (mg)	total kinase[b]	specific activity[c]	kinase yield[d]
thylakoids	92	469	278	13600	29	100
PS II-enriched membranes	9	59	24	3360	57	25
LHC II	5	13	19	143	11	1
PS II core complexes	8	18	4	2950	164	22

[a] Fractionation of spinach thylakoids and assays of protein, chlorophyll, and protein kinase activity were carried out as described in Experimental Procedures. For histone kinase assays samples equivalent to 20 μg of protein were incubated with 20 mM Tricine (pH 8.0), 0.5 mg of histone III-S/mL, 5 mM DTT, 10 mM $MgCl_2$, 0.5 mM ATP, 3 μCi of [γ-^{32}P]ATP at 22 °C for 10 min. After spotting on Whatman 3MM paper and washing to remove unbound phosphate, incorporated radioactivity was measured by Cerenkov counting. [b] Picomoles of phosphate incorporated into histone III-S/min. [c] Picomoles of phosphate incorporated into histone III-S/min/mg of protein. [d] Relative to total kinase activity of thylakoids.

RESULTS

Solubilization of spinach thylakoids with OG yields an active protein kinase extract and a comparably active residuum (data not shown). The OG extract contains at least 20 proteins, of which few, if any, bind chlorophyll (Race et al., 1995a). The unsolubilized membranes retain the bulk of the PS I and PS II components. A more exhaustive fractionation protocol was adopted to determine whether the kinase activity in the unsolubilized membranes was distinct in its protein origin from that solubilized with OG. Exposure to TX-100 releases the appressed regions of the thylakoid membrane, which form PS II-enriched vesicles (Berthold et al., 1981). The data in Table 1 demonstrate that at least one protein kinase copurifies with the PS II-rich fraction. Subsequent treatment with OG at high ionic strength resolves these vesicles further, separating the bulk of LHC II proteins from core complexes of PS II, which are devoid of membrane structure but retain some lipid together with ca. 10 proteins in the molecular weight range 5−50 kDa (Ghanotakis et al., 1987). Components associated with the water:PQ oxidoreductase activity of PS II also remain bound, but virtually all LHC II is removed. Analysis of specific protein kinase activities in these fractions demonstrates that the enzyme, hereafter denoted as PS II−PK, copurifies with the PS II core complex. When supplied with exogenous substrates, PS II−PK phosphorylated histone III-S at 4−6 times the rate obtained with casein, phosvitin, histone VIII-S, or myosin light chains (data not shown). Histone III-S has previously been shown to compete with LHC II for phosphorylation by the *in membrano* kinase activity (Coughlan et al., 1988). Interestingly, only very low levels of kinase activity fractionate with the dominant thylakoid phosphoproteins: the 25- and 27-kDa LHC II polypeptides.

The ability of PS II−PK to phosphorylate endogenous thylakoid substrates was analyzed using SDS−PAGE followed by autoradiography to detect radioactively labeled proteins obtained after incubation with [γ-^{32}P]ATP. Phosphorylation of the 43-kDa chlorophyll *a*-binding antenna protein occurs within 3 minutes of exposure of PS II core complexes to [γ-^{32}P]ATP (Figure 1*a*, lane 1). Restoration

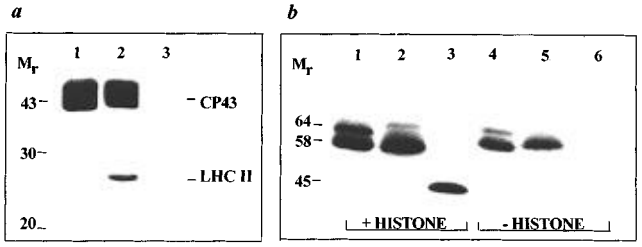

FIGURE 1: Phosphorylation of endogenous proteins and reactivation of protein kinase activity in PS II core complexes. (*a*) PS II core complexes and purified LHC II proteins were incubated with [γ-^{32}P]-ATP for 3 min before termination of phosphorylation and electrophoresis on a 13.5% polyacrylamide/4 M urea gel. Lane 1, PS II complexes; lane 2, PS II complexes + purified LHC II; lane 3, purified LHC II. Positions of molecular weight standards and major phosphoproteins are indicated. (*b*) OG extract (lanes 1 and 4), PS II complexes (lanes 2 and 5), and catalytic subunit of cAMP-dependent protein kinase (lanes 3 and 6) were electrophoresed on a 10% polyacrylamide gel before transfer of the proteins to PVDF. Renaturation was followed by incubation with histone for 1 h where indicated. The filters were treated with [γ-^{32}P]ATP for 30 min before washing to remove unincorporated label. Positions of major phosphoproteins are indicated.

of purified LHC II proteins to the PS II core complexes results in their phosphorylation within the same time frame (Figure 1*a*, lane 2). Phosphorylation is not seen when LHC II proteins are incubated with nucleotide in the absence of PS II (Figure 1*a*, lane 3). The D1 and D2 reaction center proteins, the PsbH protein (also known as the 9-kDa phosphoprotein) and a 5-kDa protein (Webber et al., 1989), are phosphorylated at longer incubation times (data not shown), in keeping with the pattern described for thylakoid membranes (Bennett, 1979).

Electrophoretic fractionation of proteins followed by immobilization and renaturation of activity (San Agustin & Witman, 1995) in the presence of an artificial substrate, histone III-S, was employed to estimate the molecular mass of PS II−PK. Two putative protein kinases, with masses of 64 and 58 kDa, are apparent in the OG extract of thylakoids (Figure 1*b*, lane 1). The PS II core complexes (Figure 1*b*, lane 2) are dominated by the 58-kDa renatured protein but retain a low level of activity around 64 kDa. The catalytic subunit of cAMP-dependent protein kinase is also renatured under these conditions (Figure 1*b*, lane 3). Chasing with unlabeled ATP did not diminish the amount of radioactivity associated with any of the phosphoproteins (data not shown), indicating that there is covalent attachment of phosphate to protein rather than electrostatic interaction between ATP and a binding site. If incubation with histone III-S is omitted, the pattern of radiolabeling is unchanged (Figure 1*b*, lanes 4 and 5) and the lower level of phosphate incorporation presumably originates from autophosphorylation of the kinase proteins. The catalytic subunit is not subject to autophosphorylation (Erlichman et al., 1974), and no labeling is apparent in the absence of histone (Figure 1*b*, lane 6).

An intimate association between PS II−PK and the reaction center of the photosystem is confirmed by the enzyme's apparent sensitivity to light. The impairment of oxygenic photosynthesis as a result of excessive irradiance is currently considered a two-step process manifest as a reduced efficiency of PS II electron transport followed by degradation of various protein components of the photosystem (Aro et al., 1993). Exposure of the PS II core complexes

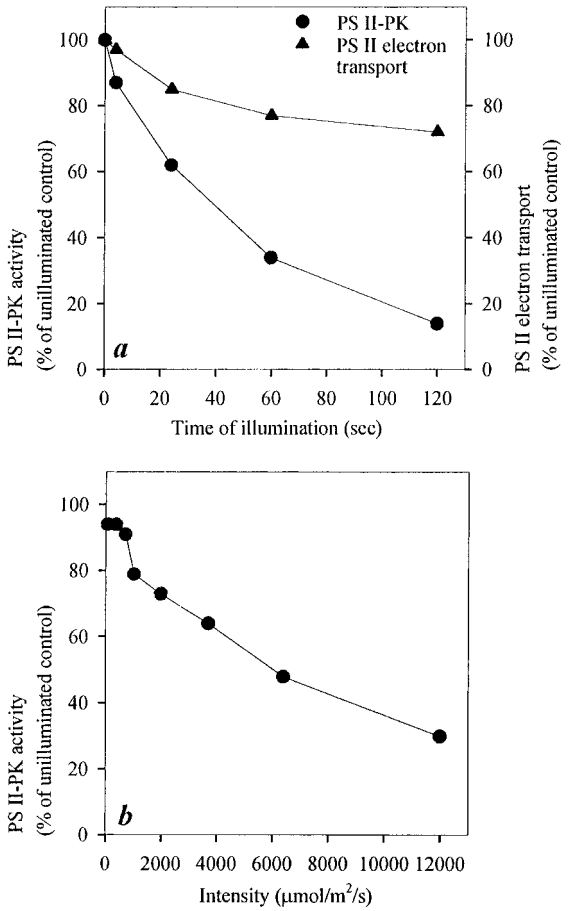

FIGURE 2: Photoinactivation of PS II−PK and electron transport in PS II core complexes. (*a*) Time course of inactivation of PS II core complexes (230 μg/mL) resuspended in 50 mM Hepes (pH 7.5), 10 mM NaCl, 5 mM $CaCl_2$, 1 mM DTT and illuminated with red light (12 000 μmol/m^2/s). Aliquots were removed at the times indicated, stored on ice, and assayed within 10 min. Protein kinase activity was measured by 10 min incubation with 20 mM Tricine (pH 8.0), 0.5 mg of histone III-S/mL, 10 mM $MgCl_2$, 0.5 mM ATP, 5 mM DTT, 3 μCi [γ-^{32}P]ATP. After termination of phosphorylation, samples were spotted on Whatman 3MM paper and washed to remove unbound label. Incorporated radioactivity was determined by Cerenkov counting. Electron transfer kinetics of PS II core complexes (8.5 μg of Chl/mL) were assayed in the presence of 1,5-diphenylcarbazide (1.5 mM) and 2,6-dichlorophenolindophenol (50 μM) in 50 mM Tris (pH 7.5), 10 mM NaCl, 5 mM $CaCl_2$ using an Aminco DW2000 spectrophotometer. Control values in unilluminated samples were 133 pmol of phosphate incorporated into histone III-S/mg of protein/min and 60 μequiv/mg of Chl/h, $n = 3$ or 4. (*b*) PS II−PK activity in core complexes after 1 min illumination with varying intensities of red light. Aliquots were processed and protein kinase activity was measured as described in *a*. The control value for unilluminated samples was 172 pmol of phosphate incorporated into histone III-S/mg of protein/min, $n = 4$.

to intense red light (12 000 μmol/m^2/s) resulted in a rapid loss of protein kinase activity toward endogenous substrates (data not shown). In order to distinguish the effect of high irradiance on PS II−PK from possible destructive modifications to its endogenous substrates, kinase activity was assayed using the exogenous substrate histone III-S. This revealed a half-time for the inactivation of ~40 s (Figure 2*a*). Measurement of PS II electron transport in equivalent samples demonstrates that this is markedly less sensitive to illumination (Figure 2*a*). No changes in polypeptide composition were observed to result from these short illumination periods (data not shown). Loss of PS II−PK activity is

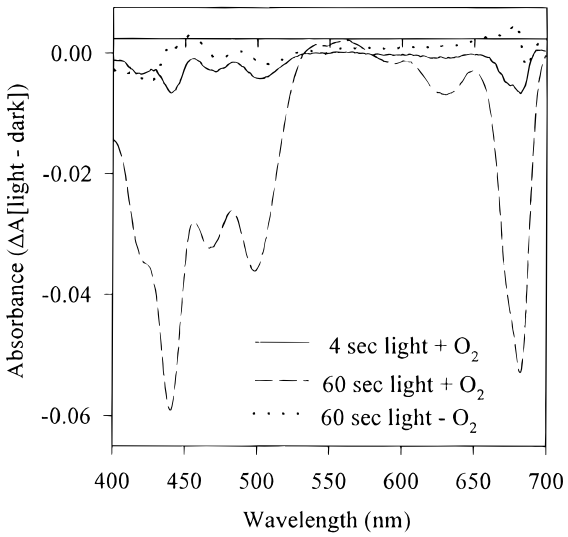

FIGURE 3: Photobleaching of pigments in PS II core complexes after red light illumination in the presence and absence of oxygen. PS II core complexes (230 μg/mL) were resuspended in 50 mM Hepes (pH 7.5), 10 mM NaCl, 5 mM $CaCl_2$, 1 mM DTT and illuminated with red light (12 000 μmol/m^2/s). Anaerobicity was produced by use of argon-sparged buffer and an enzymatic scavenging system: glucose (10 mM), glucose oxidase (500 units/mL), catalase (1100 units/mL). Samples were preincubated for 5 min in darkness at 22 °C to ensure efficient scavenging.

linearly related to the intensity of red irradiation above 1000 μmol/m^2/s (Figure 2*b*). At lower intensities, a saturable inhibitory process seems also to occur, possibly involving 15−20% of the total protein kinase activity. Steady state absorbance spectra show that at high light intensity, photobleaching of chlorophyll *a* and carotenoids accompanies kinase inactivation (Figure 3).

Exogenous electron acceptors and donors of PS II are believed to minimize the accumulation of potentially damaging radical species at either end of the redox chain by supplementing the electron supply at the site of water oxidation or through ensuring efficient removal of electrons from the bound quinones (Aro et al., 1993). Preincubation with the acceptor DBMIB slightly inhibits the kinase but confers some protection on the enzyme during subsequent light treatment (Table 2). When PS II core complexes are illuminated under anaerobic conditions there is no light-dependent loss of kinase activity (Table 2), although the oxygen-scavenging system used results in a 50% inhibition of the dark control rate. Anaerobic conditions also prevent the photobleaching of chlorophylls and carotenoids in PS II cores (Figure 3), as previously reported for other PS II preparations (Aro et al., 1993) . Further evidence that the inhibitory process involves an oxidant generated close to a sensitive site on PS II−PK stems from the observation that histone phosphorylation catalyzed by cAMP-dependent protein kinase is not inhibited when its catalytic subunit and PS II core complexes are illuminated together (Table 2). The site of oxidant formation is presumably also inaccessible to superoxide dismutase (Table 2). The mechanisms underlying inhibition of kinase activity in unilluminated samples treated with DBMIB or with radical scavengers are not clear. Omission of DTT does not lead to enzyme inactivation within the assay period.

The tight physical association between PS II−PK and the reaction center core is also apparent upon treatment of PS II complexes with 0.8 M Tris (Ghanotakis et al., 1989) (Table 2). Removal of the 16-, 22-, and 33-kDa extrinsic proteins results in a slight loss of kinase activity but does not alter the susceptibility of the remainder to photoinactivation. In contrast, prior phosphorylation of the complex inhibits the histone kinase activity of PS II−PK by 63% (Table 2) and subsequent illumination results in a more extensive loss of the remaining activity than is observed in non-phosphorylated samples. Many protein kinases modulate their activity through autophosphorylation (Krebs & Beavo, 1979), and PS II−PK may be down-regulated in this fashion.

Table 2: Modulation of PS II−PK Activity[a]

sample	specific activity prior to illumination	histone kinase activity after 1 min illumination (% of unilluminated control)
PS II core complexes	133	44
Tris-washed complexes	103	36
phosphorylated complexes	50	19
PS II core complexes:		
+ DBMIB	99	59
− oxygen	67	97
+ superoxide dismutase	87	26
catalytic subunit of cAMP-dependent protein kinase	353[b]	98[b]

[a] Tris-washing and phosphorylation of PS II complexes were carried out as described in Experimental Procedures. PS II cores (230 μg of Chl/mL) were resuspended in 50 mM Hepes (pH 7.5), 10 mM NaCl, 5 mM $CaCl_2$, 1 mM DTT and illuminated with red light. DBMIB (0.2 mM), superoxide dismutase (10 units/μg of Chl) and catalytic subunit of cAMP-dependent protein kinase (0.018 μg of protein/μL, 1.0 unit/μg of protein) were preincubated with the PS II complexes for 5 min in darkness at 22 °C. DTT was excluded when DBMIB was used. Anaerobicity was produced by use of argon-sparged buffer and preincubation with an enzymatic scavenging system: glucose (10 mM), glucose oxidase (500 units/mL), catalase (1100 units/mL). 20 μL samples were incubated with 20 mM Tricine (pH 8.0), 0.5 mg of histone III-S/mL, 5 mM DTT, 10 mM $MgCl_2$, 0.5 mM ATP, 3 μCi of [γ-^{32}P]ATP at 22 °C for 10 min. Incorporated radioactivity was measured by Cerenkov counting of samples spotted on Whatman 3MM paper. [b] Values were calculated by subtraction of PS II−PK activity from total histone kinase activity in sample containing PS II complexes and catalytic subunit of cAMP-dependent protein kinase.

DISCUSSION

This is the first reported phosphorylation of both PS II and LHC II proteins by a fraction purified from thylakoid membranes. The copurification of PS II−PK with the core complex of the photosystem can be rationalized by assuming the diffusional constraints of the membrane require a close physical proximity between the enzyme and its substrates if phosphorylation is to occur on a functionally significant time scale. The differing positions and contexts of the modified threonine residues within substrate polypeptides were previously considered indicative of a multikinase system (Allen, 1992) with one enzyme targeted toward the LHC II proteins and a second directed toward the remaining PS II substrates. The substrate preference of PS II−PK appears sufficiently broad for residues in both types of environment to be phosphorylated *in vitro*. Our data do not preclude the existence of additional kinases operating on some or all of these substrates *in vivo*. Moreover, the existence of distinct populations of LHC II with differential levels of phosphorylation (Larsson et al., 1987; Peter & Thornber, 1991) suggests that more than one kinase is involved in the modification of these proteins.

The identification, using the renaturation blot assay technique, of a 58-kDa protein as the putative PS II−PK confirms that the enzyme is distinct and novel. This assignment is based upon the correlation between increasing levels of 58-kDa protein and kinase activity which are observed upon solubilization of thylakoids to produce PS II-enriched membranes and PS II core complexes. The amount of 64-kDa protein associated with the preparations decreases during the fractionation procedure. No 58-kDa polypeptide component has previously been identified in the PS II core complex either in stoichiometric or substoichiometric abundance, and no identified PS II protein contains the consensus motifs which characterize protein kinases (Hanks et al., 1988). It seems reasonable to conclude that PS II−PK is substoichiometric with respect to the PSII core. An efficient exchange of such a tightly associated kinase between the cores of adjacent photosystems seems improbable, hence we speculate that there exists a specialized minority population of kinase-containing PS II complexes. These may be strategically located at the margins between the appressed and non-appressed thylakoids, so as to facilitate the detachment of LHC II after phosphorylation.

The detection of additional protein kinase activities in the membrane extracts is not unexpected as up to 13 thylakoid proteins are evidently subject to phosphorylation (Silverstein et al., 1993). A 64-kDa protein was previously purified from a detergent extract of thylakoids using an ATP-analog affinity matrix, although the eluted product did not retain enzyme activity (Race et al., 1995b). There is an extensive earlier literature regarding a protein kinase of 64-kDa (Coughlan et al., 1988; Gal et al., 1990) which has been termed an LHC II kinase (Gal et al., 1990), though preparations enriched with this enzyme did not catalyze the phosphorylation of LHC II proteins at a significant rate. Regulation of this kinase through an interaction with a PQ-binding site on the cytochrome b_6f complex has been proposed (Frid et al., 1992). It is noteworthy that the 64-kDa kinase and the cytochrome complex cofractionate into OG, whereas PS II core preparations lack cytochromes b_6 and *f*. We have been unable to demonstrate redox control of PS II−PK thus far, but the redox sensitivity of all thylakoid protein phosphorylations has been documented (Bennett, 1991). Hence we may reasonably speculate that the activity of PS II−PK is sensitive to the redox status of PQ bound to the D1 or D2 polypeptides in the PS II core. A critical examination of redox influence on individual endogenous protein phosphorylations *in membrano* could test this hypothesis.

PS II−PK is evidently a prime, although presumably indirect, target for photoinactivation in agreement with the thesis that the enzyme is intimately associated with those redox components of the photosystem which have been postulated to be involved in formation of reactive radical species during over-excitation of the complex (Aro et al., 1993). Photobleaching is ascribed (Asada & Takahashi, 1987) to an interaction of chlorophyll triplet states with molecular oxygen that produces singlet oxygen and hydroxyl radicals, which promote irreversible damage to photosynthetic proteins and pigments (Aro et al., 1993). Although the photodestruction of PS II−PK may not occur in intact membranes under physiological conditions, it can reasonably be assumed to accompany any photobleaching of pigments observed *in vitro* or *in vivo*.

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