Photosystem II Particles from Chlamydomonas reinhardtii

PURIFICATION, MOLECULAR WEIGHT, SMALL SUBUNIT COMPOSITION, AND PROTEIN PHOSPHORYLATION*

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PSII particles from Chlamydomonas reinhardtii were purified according to the protocol of Diner and Wollman (Diner, B. A., and Wollman, F.-A. (1980) Eur. J. Biochem. 110, 521–526) followed by ion-exchange chromatography. They contained the psbA, psbB, psbC, and psbD gene products in a 1/1/1/1 stoichiometry, cytochrome b599, and several small polypeptides, and exhibited electron transfer from donor Z to acceptor QA (40–50 chlorophylls/reducible QA). Upon ultracentrifugation and molecular sieving in the presence of either lauryl maltoside or octaethylene glycol dodecyl ether (C&8), they behaved as monomers of 440–510 kDa, including the detergent. C12E8 preparations also contained a small proportion of a partially interconvertible dimeric form.

Four small subunits were identified by N-terminal sequencing, namely a 6.1-kDa nuclear-encoded subunit and three chloroplast-encoded subunits homologous to psbE, psbK, and psbM gene products. Cytochrome b599 subunit (α (psbE)) of C. reinhardtii, but not subunit β (psbF), was recognized by an antiseraum raised against higher plant cytochrome b599. The products of the psbF, psbI, and psbN genes remained undetected, presumably because of blocked N termini.

At least four polypeptides presented both phosphorylated and unphosphorylated forms (psbc, psbd, and psbl gene products, as well as an unidentified 5-kDa subunit).

Photosystem II (PSII)* carries out the light-driven electron transfer from water to plastoquinone in the photosynthetic membranes of oxygen-evolving organisms. PSII complexes from higher plants, cyanobacteria, and algae have been extensively studied. They comprise numerous prosthetic groups and a large number of subunits encoded either by chloroplast or nuclear genes. The function of many of these polypeptides remains unknown (reviewed by Ghanotakis and Yocum, 1990).

The simplest photoactive PSII reaction centers yet obtained in higher plants contain five intrinsic subunits encoded in the chloroplast by psbA, psbD, psbE, psbF, and psbI genes (Nanba and Satoh, 1987; Webber et al., 1989). psbA and psbD encode the two larger subunits, respectively D1 and D2, which cooperate in the binding of the primary reactants. These subunits show sequence homologies with subunits L and M of the purple bacteria reaction centers (Michel et al., 1986; Trebst, 1996). The three other subunits are small, and each probably forms a single transmembrane α-helix: psbE and psbF encode subunits α and β of cytochrome b599, respectively (Herrmann et al., 1984), whereas the product of the psbl gene is not known to bind any cofactor.

Larger PSII complexes contain additional subunits, many of which are involved in energy collection or oxygen evolution. The "core antenna" is comprised of two intrinsic subunits encoded in the chloroplast by the psbB and psbC genes (Bricker, 1990). A major light-harvesting complex (LHClI) and minor chlorophyll-protein complexes (CPmin) are present in PSII membranes but essentially absent in PSII particles (Ghanotakis and Yocum, 1990; Bassi and Dainese, 1990); they are comprised of nuclear-encoded polypeptides that bind chlorophylls a and b. Three extrinsic subunits (OE1, OE2, and OE3), encoded by nuclear genes, are part of the oxygen evolution center: the smaller oxygen-evolving PSII particles contain OE1 but neither OE2 nor OE3 (e.g. see Ghanotakis et al. (1987) and Haag et al. (1990)). Several other subunits (such as those encoded in chloroplasts by the psbh, psbJ, psbK, psbL, psbM, and psbN genes) have been identified (generally by N-terminal sequencing) in PSII particles from various species (Ikeuchi et al. 1989a, 1989b, 1989c; Koike et al., 1989; Webber et al., 1989).

The unicellular green alga Chlamydomonas reinhardtii is a good model system for studying PSII because of the possibility of growing the cells heterotrophically, of obtaining photosynthesis mutants, of transforming cells with foreign DNA, and of studying nucleus-organelle interactions. Several C. reinhardtii polypeptides, some of them phosphorylated, have been proposed to be PSII subunits on the basis of their presence in PSII particles and their absence in PSII-deficient mutants (Delepelaire, 1984; Delepelaire and Wollman, 1985; de Vitry et al., 1987). Although the genes encoding the large subunits of PSII have been identified and sequenced, little is known about the small subunits. One aim of the present study is to further characterize PSII subunits in C. reinhardtii and to...
establish to what extent these subunits are common to higher plants and cyanobacteria. We describe the isolation of PSII particles from *C. reinhardtii*, further purified with respect to the earlier procedure (Diner and Wollman, 1980). We have analyzed the particle composition (components and stoichiometry). We show that the molecular weight of the complex in two detergents (lauryl maltoside and C12E9) corresponds to that of a monomer. We have characterized several small PSII subunits by N-terminal sequencing, immunoblotting, pulse-labeling in the presence of translation inhibitors, and 32P-labeling of phosphopolypeptides.

**MATERIALS AND METHODS AND RESULTS**

**Discussion**

Purification and Molecular Weight of PSII Particles—Further purification of photoactive PSII particles from *C. reinhardtii* was achieved by following the previous procedure (Diner and Wollman, 1980) with an ion-exchange chromatography step. This step removes the extrinsic proteins and most of the peripheral antenna without inactivating the reaction center. The monodispersity of the preparations was established by HPLC gel molecular sieving. The particles are similar in photoactivity (electron transfer from secondary donor Z to primary quinone acceptor Qa, with a stoichiometry of 40-50 chlorophylls/reducible Qa) in subunit composition, and in size (without detergent) to previously described preparations of non-02-evolving PSII particles from cyanobacteria (Rögner et al., 1990; Dekker et al., 1988) and higher plants (Akabori et al., 1988). Such particles are sometimes referred to as PSII cores.

Taking into account the contribution of two small subunits whose presence is highly probable but which remained undetected, presumably because of blocked N termini (psbF and psbI gene products), the total M, of identified proteins and cofactors in *C. reinhardtii* PSII particles (see below) is ~274,000 (Table 1A). This value is slightly higher than that of 255,000 (without detergent) estimated for *Synechococcus* particles on the basis of gel filtration (Rögner et al., 1990; Schroder et al., 1988) and higher plants (Webber et al., 1989) but not in cyanobacteria (Ikeuchi et al., 1989c). We attempted to establish by immunoreaction the presence of the β subunit, but the antisemur we used, raised against spinach cytochrome bo3, recognized only the α subunit in *C. reinhardtii*.

The composition in low molecular weight subunits of *C. reinhardtii* PSII particles therefore appears consistent with that observed in other organisms. Except for cytochrome bo3, none of these small polypeptides has been shown to bind cofactors, and their role is still unknown. Given the small size of the corresponding genes, the probability of nondirected mutations is low. The possibility to disrupt or modify these genes by homologous recombination should give clues as to how essential they are for the function and assembly of the PSII complex.

Many small subunits present in PSII particles are predicted to form a single transmembrane α-helix with short extramembrane segments (e.g. psbM in Table 3). Altogether, 10 or 11 different subunits of this type have been characterized (see Table 2). This process of “microassembly” (de Vitry and Popot, 1988; Popot and de Vitry, 1990) is exemplified by the proposed structure of cytochrome bo3, in which two independent subunits, each forming a single transmembrane helix, contribute 1 histidine residue each toward ligation of the heme (Herrmann et al., 1984). The existence of such subunits reflects the strong conformational constraints to which transmembrane polypeptides are subjected. As the larger folding domains encountered in soluble proteins, single transmembrane helices can fold autonomously, providing specific surfaces for association with the rest of the complex (Popot and Engelman, 1990).

**PSII Phosphorylation**—At least three PSII proteins can be phosphorylated, namely P6 (product of the psbC gene, which yields P6’), D2.2 (psbD product, yielding D2.1), and L7 (presumably psbH product, yielding L5 and L6). The apparent M, in SDS-urea-PAGE gels of the nonphosphorylated forms was lower than that of the phosphorylated ones. Their amount increased with dephosphorylation by phosphatase, paralleling the decrease of the phosphorylated forms. They were still present in PSII mutants devoid of PSII phosphorylation, whereas the phosphorylated forms were absent.

The existence of slow posttranslational phosphorylations leading to the appearance of P6’, D2.1, L5, and L6 was first established by Delepelaire (1984). In his 14C-labeled pulse-chase experiments, appearance of the phosphorylated forms paralleled a decrease in the nonphosphorylated proteins P6, D2.2, L7, and L3. Our data suggest that L7 is the nonphosphorylated form and that L5 and L6 are two different phosphorylated forms of the psbH gene product. However, we

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2 Portions of this paper (including “Materials and Methods,” “Results,” Figs. 1–6, and Tables 1–5) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
cannot exclude that L5, L6, and L7 correspond to more than one protein.
The N-terminal sequence of psbH product in C. reinhardtii is aligned with that in other species in Table 4. The second residue (Thr-2) has been shown to be phosphorylated both in C. reinhardtii (Dedner et al., 1988) and in spinach (Michel and Bennet, 1987). The existence of two distinct phosphorylated forms, L5 and L6, suggests that in C. reinhardtii other residue(s) could also be phosphorylated. Thr-17 is a possible candidate. Phosphorylation of Thr-17 would be consistent with the low yield of PTH-derivative observed for this residue, similar to that for Thr-2, in the N-terminal sequence analysis of Dedner et al. (1988). Distinct forms of phosphorylation of the psbH gene product have not been detected previously in other organisms, possibly because of limited resolution of the gels.

The existence of one (or more) phosphopolyopeptide(s) migrating with an apparent M, of about 5,000 has already been reported in C. reinhardtii (de Vitry et al., 1987) and wheat (Webber et al., 1989). In the latter case, it was proposed to originate from phosphorylation of the psbL gene product on Thr-2. The sequence of C. reinhardtii psbL, aligned with that of other species in Table 5, does not include Thr-2. All of its threonine and serine residues are located very close to, if not within, the putative transmembrane segment, and their accessibility to kinases may be limited. It seems possible that the psbL protein may not be phosphorylated in C. reinhardtii, but rather that the centers in these dimers cooperate is not known. Spectroscopic accessibility to kinases may be limited. It seems possible that the centers in these dimers cooperate is not known. Spectroscopic

In conclusion, we have isolated purified PSII particles from C. reinhardtii. Their polypeptide and pigment composition is very similar to that of higher plant PSII particles, as well as their complement of phosphopolyopeptides. Depending on the detergent, the particles are either totally or mainly monomeric. Indirect evidence suggests that dimers may be an artefact of isolation and not naturally occurring. The similarity of C. reinhardtii and higher plant PSII complexes confirms the usefulness of this unicellular green alga as an organism in which to study the structure, assembly, and regulation of PSII, using combined biophysical, biochemical, and genetic approaches.

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C. reinhardtii Photosystem I1 Particles

Supplemental Material to: Photosystem I1 particles from Chlamydomonas reinhardtii: preparation, molecular weight, small subunit composition, protein phosphorylation.

By Catherine de Vitry, Bruce A. Diner and Jean-Luc Pogot

MATERIALS AND METHODS

Cell culture conditions and mutants

WT and mutants of C. reinhardtii were grown at 50 lx in Tri-citate-phosphate medium, pH 7.2. PSI particles were isolated from the double mutant F34-14, which lacks photosystem I and chloroplast ATP synthase (Diner et al., 1980; de Vitry et al., 1989). PSI1 mutants F34 (lacking synthesis of Psbc product) and F387 (lacking synthesis of psbA product) show PSI1 phosphorylation (Benoun et al., 1986; Deleparse et al., 1984; de Vitry et al., 1989).

Protein isolation

PSI1 thylakoid membranes were isolated as described (Brown & Benoun, 1977). PSI1 was purified from F34-14 thylakoid membranes solubilized in 1% digitonin (Merck) in a choline concentration of 1.2 mg/ml during 1 h at 4°C. PSI1 particles were separated by sucrose density gradient centrifugation (Brown & Benoun, 1980). A column of DEAE-Sepharose Fast Flow (Pharmacia) was equilibrated with buffer A (10 mM Tris pH 8.0, 0.1% lauryl sarcoslate (LS), 200 mM NaF). The gradient containing the PSI1 particles was diluted 5-fold in buffer A (0.5 mg/ml Tris pH 8.0, 0.1% lauryl sarcoslate (LS), 200 mM NaF) and loaded on the column. The column was washed first with buffer A, then with buffer A plus 100 mM NaCl in order to detect the bands located in the gradient and to obtain PSII particles. PSI1 particles were isolated from 25 ml of thylakoid membranes in 300 ml of buffer A containing 200 mM NaF and collected on a sucrose cushion in buffer A plus 200 mM NaCl. PSI1 particles were separated from PSI2 particles by sucrose density gradient centrifugation (Diner & Wollman, 1980).

Materials and Protocol

Materials and Methods

Phosphate medium. pH 7.2. PSI1 particles were isolated from the double mutant WT and mutants of C. reinhardtii were grown at 300 lux in Tris-acetate-F54-14, which lacks photosystem I and chloroplast ATP synthase (Diner et al., 1980; de Vitry et al., 1989). PSI1 mutants F34 (lacking synthesis of Psbc product) and F387 (lacking synthesis of psbA product) show PSI1 phosphorylation (Benoun et al., 1986; Deleparse et al., 1984; de Vitry et al., 1989).

UPLC Material

To purify PSI1 particles, PSI1 membranes were solubilized in 1% choline chloride. Under these conditions, PSI1 particles were isolated from 15 ml of thylakoid membranes after 1 h of centrifugation at 100,000 g for 1 h at 4°C. PSI1 particles were then isolated as described above.

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HPLC purification

The concentration of PSI1 reactions centers was determined by measuring absorption at 650 nm. The amount of PSI1 particles was determined by the absorption of PSI1 particles at 650 nm.

Other reactions

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RESULTS

1. Molecular weight of purified PSI1 particles

1.1. Molecular weight of the monomeric form

C. reinhardtii PSI1 particles prepared according to the procedure of Simon & Wolman (1989) were adsorbed on a DEAE-Sephacel column. Washing with a buffer containing 0.1 M NaCl detached most of the extrinsic polypeptides and of the peripheral antenna. The PSI1 particles were then eluted with a buffer containing 0.2 M NaCl. All steps were done in the presence of 0.03% LM. The particles were collected by centrifugation after dilution below the one of LM and frozen. For analysis, the particles were thawed and resuspended into either 0.03% LM or 0.03% C,2E, solution, as needed (see Materials & Methods).

Particles resuspended in LM appeared monodisperse upon HPLC molecular sieving (Fig. 1). Under actinic illumination, they showed active electron transfer from the secondary donor D (a tyrosine residue of subunit D1; Schell et al., 1988; Nets et al., 1989) to the primary quinone acceptor QA, with a stoichiometry of 45-50 chlorophyll per reducible QA (data not shown).

The molecular weight of the PSI1/detergent particles in either LM or C,2E, resisted their elution from the column or from their diffusion coefficient, and their sedimentation coefficient (Table IB). Densities were measured by equilibrium sedimentation, diffusion coefficients by filtration on a molecular sieve HPLC column and sedimentation coefficients by rate-zonal centrifugation. Combining these data yields molecular weights of 440,000 in LM and 510,000 in C,2E, as shown below, these values are compatible only with monomeric complexes, containing one reaction center per particle.

1.2. Occurrence of a dimeric form

The occurrence of C,2E, a small proportion of a heavier form was detected by sedimentation velocity centrifugation (heavy/heavy-light: 17 ± 4; 3 determinations). The sedimentation coefficient of the heavier form corresponded closely to that expected for a dimer of the light form (Table 1B). The polypeptide compositions of the two forms were similar when analyzed by CBB-stained SDS-PAGE gels. Dimers and monomers had the same absorption spectrum and the same fluorescence induction curves in presence of an artifical donor (dicyclohexylcarbodiimide). They had the same photoactivity and their induction curves were superimposable, indicating absence of cooperation between the two centers present in the dimer. They broadened somewhat the same amount of C,2E, per chlorophyll (see below).

Incubation of the particles with a high concentration of the reductant dithiotreitol (DTT; 250 mM; 4-14 h, 4°C) prior to sedimentation velocity analysis diminished the proportion of dimers to about 2/3 its value in controls. When the monomeric form, separated either after or without incubation with DTT, was collected, dialyzed to remove excess DTT, concentrated by ultrafiltration on Centricon 30 and analyzed again on sucrose gradients, reformation of some dimeric form was observed. These interconversions confirmed the monomer/dimer relationship and suggested that dimerization somehow involved the formation of disulfide bridges. Further experiments however did not support the latter hypothesis.

Namely: 1) the DTT-treated reduced monomer with indocarbocyanine (I-100 nm) or Dicyclohexylcarbodiimide (DNB; 1.5 mM), in order to block free sulfhydryls by alkylation, did not prevent reformation of the dimer; 2) homogenization of the cells in the presence of DNB (1.1 mM), in order to protect preexisting disulfide bridges from artifactual reduction, did not increase the proportion of dimer in the purified PSI1 preparations. These experiments therefore gave few clues as to the molecular mechanism of dimerization in vitro, and we do not present to draw inferences about the existence of PSI1 reaction center dimers in vivo.

2. PSI1 particle composition

As PSI1 subunit names and N, differ from one organism to the next, nomenclature tends to be confusing. The denomination and principal characteristics of PSI1 subunits in C. reinhardtii are summarized in Table 2, together with information gathered in the present work about their presence or absence in purified polypeptides and the occurrence of phosphorylated forms (see below).

Table 2: PSI1 subunits and their characteristics. Subunit presence in PSI1 particles is indicated + when present, − when most certainly present but not detected, probably because of blocked N-terminus, × when scarce, 0 when absent, nd when not determined. Gene nomenclature is generally accepted. Apparent molecular weight (kDa) in SDS-urea gel is indicated for C. reinhardtii; when the subunit has not been identified in C. reinhardtii, M, in other organisms is given between parentheses. * indicates subunits whose N-terminal has been sequenced in the present study. Protein nomenclature varies widely among species and is indicated for non-phosphorylated (nom-phos') and phosphorylated (phos') forms of C. reinhardtii only. DPP: small phosphopolypeptide(s). Phosphorylation in higher plants (phos') is indicated + , − , + or − when subunit might be phosphorylated. K, number of putative transmembrane a-helices; hydrophobic subunits that have not yet been totally sequenced are indicated i (integral), a-iP, N-terminus residues are alanine followed by a phosphothreonine.

2.1. Large subunits

SDS-PAGE analysis of PSI1 particles revealed the presence of four polypeptides larger than 10 kDa and three bands (A-C) of small molecular weight (Fig. 2). The four large polypeptides correspond to the two core antenna subunits (called PsbF and PsbE), encoded in the chloroplast by the psbE and psbF genes, and two reaction center subunits (D1 and D2) encoded by chloroplast genes psbD and psbD (Scheiha & Erickson, 1989). Gene products of psbC and psbD appear as double bands corresponding to a phosphorylated and a non-phosphorylated form (PsbC and PsbD, D1 and D2) in vivo below.

N.W., N-terminal residues are alanine followed by a phosphothreonine.
The absence of the three oxygen-evolving enhancers in the PSI particles is shown in CBB-stained SDS-PAGE gels (Fig. 2) and was confirmed by the absence of reaction with antibodies against OEE1, OEE2 and OEE3 of C. reinhardtii (results not shown). The absence of most of the peripheral and minor antenna subunits is shown in CBB-stained SDS-PAGE gels (Fig. 2) and in autoradiograms of 35S-labelled PSI particles (Fig. 3). None of the bands P9, P11, P14 and P17 were present. Presence of some chl a/b-binding antenna is suggested by the chl a/b ratio in the PSI particles (1.3:1, 3 preparations). The stoichiometry of the four large polypeptides was estimated to be 1:1:1:1 by densitometric scanning of autoradiograms of stationary 35S-labelled PSI particles (Fig. 3; see de Vitry et al., 1987).

2.1. Small subunits: 6.1-kDa subunits, pab6, pab8 and pab9 gene products

In order to identify the small subunits and compare them with those of other species, N-terminal sequencing was performed on the three bands of small molecular weight. In Table 3, the N-terminal sequences obtained are aligned with the N-terminal sequence of mature proteins or predicted precursors from other species.

Nuclear-encoded 6.1-kDa

| Species       | Ref.     | Sequence                        |
|---------------|----------|---------------------------------|
| Tobacco       | c        | MRRVVRVEENVKTVSQND...           |
| Rice          | d        | MRVRVYQVDNLYVTVQVQND...         |
| Spinach       | e        | MRRVVRVEENVKTVSQND...           |
| Wheat         | f        | KLPEAYAPFAPIVDVLPVIFVFPILLAFWQAAVSPR |

The size of the cytochrome b6/f, subunit 6.1 kDa. The cytochrome b6/f subunit is conserved with respect to the first sequence listed. *: sequence is interrupted because portions of the protein have not been further sequenced or because the alignment is limited to the extent of sequence determined in C. reinhardtii. #: N-terminal sequence determined in the present study. (M): nomenclature for translation initiation is probably missing in the mature protein. (pre): N-terminal of the mature protein has not been determined; the gene product includes a probable presequences. Predicted transmembrane a-helices are overlined. References: a, De GVita et al. (1993a); b, Cushman et al. (1986); c, Caccamo & Bryant (1989); d, De GVita et al. (1988); e, De GVita et al. (1986); f, Splitt et al. (1989); g, Bhattacharya et al. (1986); h, Willey et al. (1983); i, Splitt et al. (1989); J, Hirata et al. (1990); k, Slik et al. (1990); l, Poulova et al. (1989); m, Zhang et al. (1990); N, Stierewalt & Bryant (1989); o, Wolfe et al. (1989); p, Iwakura et al. (1989).

Band A contained two subunits with free N-termini, one of them corresponding to the nuclear-encoded subunit of 6.1 kDa found in PSI particles of higher plants and the other to a subunit encoded in the chloroplast by a plastidic gene. Judging from the ratio of the PFN derivatives, the two subunits are located within the nucleus of C. reinhardtii and the spinach 6.1-kDa subunits have a very different (8 conserved residues out of 12) Table 3). The identification of the product bound in band A was confirmed by cross-reaction with an antiserum raised against spinach cytochrome b6/f (Fig. 4). In a test experiment using pea PSI2 PSI2 particles, the antiserum revealed the a subunit of cytochrome b6/f and, more weakly, the d subunit (Fig. 4b). Longer exposures were required to reveal C. reinhardtii b56 subunit (Fig. 4b), confirming that its apparent molecular weight (6,000) is indeed smaller than observed in higher plants (9,400). The b subunit of C. reinhardtii, which had not been identified by N-terminal sequencing, was not detected either by the antiserum.

Band B contained a protein with a largely blocked N-terminus, as indicated by the very low amounts of PFN derivatives recovered. Only eight residues could be determined, half of them being conserved in the PSI2 subunit encoded by chloroplast gene psbM in higher plants (Table 3).

Table 3: N-terminal sequences of C. reinhardtii PSI2 particle nuclear-encoded subunit 6.1-kDa and subunits encoded in the chloroplast by the pab6, pab8 and pab9 genes. Alignment with N-terminal of native proteins or gene product sequenced from other species. *: sequence is interrupted because portions of the protein has not been further sequenced or because the alignment is limited to the extent of sequence determined in C. reinhardtii. #: N-terminal sequence determined in the present study. (M): nomenclature for translation initiation is probably missing in the mature protein. (pre): N-terminal of the mature protein has not been determined; the gene product includes a probable presequence. Predicted transmembrane a-helices are overlined. References: a, De GVita et al. (1993a); b, Cushman et al. (1986); c, Caccamo & Bryant (1989); d, De GVita et al. (1988); e, De GVita et al. (1986); f, Splitt et al. (1989); g, Bhattacharya et al. (1986); h, Willey et al. (1983); i, Splitt et al. (1989); J, Hirata et al. (1990); k, Slik et al. (1990); l, Poulova et al. (1989); m, Zhang et al. (1990); N, Stierewalt & Bryant (1989); o, Wolfe et al. (1989); p, Iwakura et al. (1989).

The amount of bound detergent was determined by zone zonal centrifugation using radioactive detergents (see Materials and Methods). It was similar in Cbb3 and 1A, each PSI2 particle binding about 120 kDa of detergent. As already mentioned, the detergent used contains the same amount of Cbb3 per chlorophyll as the monomeric one. Water bound to the polar heads of the detergent (35 kDa for PSI2/1A particles) was estimated as described under Materials and Methods.

Band B contained a protein with a largely blocked N-terminus, as indicated by the very low amounts of PFN derivatives recovered. Only eight residues could be determined, half of them being conserved in the PSI2 subunit encoded by chloroplast gene psbM in higher plants (Table 3).

Figure 4: Immunoblot of PSI2 particles of C. reinhardtii (C.) and of Zea mays (Z.) with an anti-cytochrome b6/f antisera. A. The antisera reveals in Zea mays the a subunit of cytochrome b6/f and, at a lesser extent the d subunit, with respective apparent molecular weights of 9,400 and 4,400. After a 20-fold longer exposition (B), the antibody reveals also in C. reinhardtii the a subunit of cytochrome b56 with an apparent Mr of 6,000.
3. PSI phosphoproteins

Labeling of whole cells in the presence of [32P]orthophosphate showed that the following PSI-associated polypeptides are phosphorylated: P6 (psbC), D2.1 (psbD), L5, L6 and a small phosphoprotein of 5 kDa (Fig. 5).

As shown in Fig. 5, treatment with alkaline phosphatase causes the disappearance of polypeptides P6' and D2.1 and a corresponding increase of P6 and D2.2 (CBB-stained gel). This apparent conversion correlates with the disappearance of the "P"-labeling (autoradiogram). These observations strongly suggest that P6' and D2.1 are the phosphorylated forms of P6 and D2.2, respectively, in keeping with the fact that D2.1 and D2.2 have the same proteolytic digestion profile (Delepelaire, 1984). The phosphorylated form therefore could be detected on autoradiograms. They migrated with an apparent mass of 8,500 during SDS-PAGE of PSII particles (Fig. 5) and of thylakoid membranes (left of Fig. 6). L5 and L6 also remained below detection in the 32P-autoradiogram of Fig. 4, indicating that they are present in sub-stromal-matrix amounts. P6'-labeling of wild-type cells (WT) is present as an inhibitor of nuclear translation indicated that L5, L6 and L7 are all synthesized in the chloroplast (Fig. 6). L5 and L6 are absent, as are the other PSI phosphoproteins P6' and D2.1, in mutants affected in PSI phosphophydrolysis (Fig. 6, lanes PUD34 and PUD7). While L7 and the non-phosphorylated polypeptides P6 and D2.1 are present. These observations suggest that L5 and L6 are two phosphorylated forms and L7 the non-phosphorylated form of the same protein. Judging from its presence in PSIII particles, its lack of accumulation in PSIII-deficient mutants, its apparent mass of 8,500, its phosphorylatability, its synthesis in the chloroplast and its behavior as an intrinsic membrane protein, this polypeptide is a reasonable candidate for being the intrinsic phosphorylprotein encoded in the chloroplast by psbH.

Figure 5: PSI phosphoproteins. CBB stained gel and corresponding autoradiogram of 32P-labelled PSI particles, treated (+) or not (-) with alkaline phosphatase. Five major PSI phosphoproteins are 32P-labelled: P6 (psbC), D2.1 (psbD), L5 and L6 (8.5 kDa), and SPP (small phosphoprotein: 5 kDa). Antenna subunits P10 and P11 are also 32P-labelled.

Finally, and as reported previously (de Virty et al., 1987), we also observed a further, unidentified 32P-labelled band, which migrates with an apparent M, of about 5,000 and may represent one or more phosphoproteins. A similar observation has been recently described in higher plants (Webber et al., 1989).

Table 4: N-termini of 8.5-kDa phosphoproteins from C. reinhardtii and alignment with other species. Threonines and serins are absent in N-terminus of C. reinhardtii psbH gene product and only present at the border of the predicted transmembrane α-helix (overlined) suggesting they may not be phosphorylated. The threonine (underlined) had been proposed to be phosphorylated in wheat (Webber et al., 1989), in absence of evidence from cyanobacteria. References: a, From et al. (1988); c, Cantrell & Bryant (1985); e, Leitch et al. (1988).